Universitat Autònoma de Barcelona

DOCTORAL THESIS

ABSORPTION, SAFETY AND TOLERABILITY OF A TOPICAL QUINOLONE (ABSORCIÓN, SEGURIDAD Y TOLERABILIDAD DE UNA QUINOLONA TÓPICA)

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3 LIST OF ABBREVIATIONS

ABSSSI	Acute bacterial skin and skin structure infections
ADR	Adverse Drug Reaction
AE	Adverse event
AESI	Adverse events of special interest
AEMPS	Agencia Española de Medicamentos y Productos Sanitarios
API	Active pharmaceutical ingredient
BLQ	Below the lower limit of quantification
b.i.d.	Bis in die (twice daily)
BSA	Body surface area
CA-MRSA	Community-acquired methicillin-resistant *S. aureus*
CAP	Community adquired pneumonia
CNS	Central nervous system
COPD	Chronic Obstructive Pulmonary Disease
ECG	Electrocardiogram
EMA	European Medicines Agency
FDA	Food and Drug Administration
GABA	Gamma-aminobutyric acid receptor
h	Hour
hERG	Human-ether-à-go-go-related gene
IC_{50}	Half maximal inhibitory concentration
ICH	International Conference on Harmonisation
IDSA	Infectious Diseases Society of America
IMP	Investigational Medicinal Product
ISR	Incurred Sample Reproducibility
IV	Intravenous
LC-MS/MS	Liquid chromatography with tandem mass spectrometry

LOQ	Limit of quantitation
MHRA	Medicines and Healthcare Products Regulatory
MIC	Minimum inhibitory concentrations
MRSA	Methicillin Resistant Staphylococcus aureus
MRSE	Methicillin-Resistant Staphylococcus epidermidis
MSSA	Methicillin-Susceptible Staphylococcus aureus
MSSE	Methicillin-Susceptible Staphylococcus epidermidis
NA	Non-available
o.d.	Omne in die (once daily)
PDCO	Pediatric Committee
PIP	Pediatric Investigational Plan
PIS	Patient Information Sheet
PK	Pharmacokinetic
PMQR	Plasmid-mediated quinolone resistance
PSM	Phenol Soluble Modulin
PVL	Panton-Valentine leukocidin
QRDR	Quinolone resistance determining regions
SAE	Serious adverse event
SSSI	skin and skin structures infection
SIRS	Skin infection rating scale
SITL	Secondarily infected traumatic lesion
SOC	System Organ Class
TEWL	Trans-epidermal water loss
t.i.d.	Ter in die (three times daily)
USA	United States of America
vs.	Versus
WHO	World Health Organization

4 BACKGROUND

4.1 QUINOLONES

4.1.1 INTRODUCTION

Quinolones are among the most commonly prescribed class of antibacterial drugs in the world and are used to treat a variety of Gram-negative and Gram-positive bacterial infections humans both in outpatient and inpatient settings [1, 2, 3]. Quinolones have been prescribed widely to treat respiratory tract infections, including tuberculosis, urinary tract infections, intra-abdominal infections, skin and skin structure infections, sexually transmitted diseases, and bone and joint infections [4]. They have also been used for prophylaxis in neutropenic patients with cancers, in cirrhotic patients at risk for spontaneous bacterial peritonitis, and in urologic surgery [5, 6]. The quinolone antibiotics inhibit to varying degrees the bacterial enzymes DNA gyrase and topoisomerase IV, which are responsible for introducing negative supercoils into DNA that are essential in most nucleic acid processes, help control levels of DNA under- and over-winding, and remove knots and tangles from the bacterial chromosome. Therefore, these are critical enzymes for the transcription and replication processes of bacterial DNA as they are needed for, among other functions, solving the accumulated superhelical tension ahead of polymerases and decatenating the two newly formed DNA strands [7]. Both DNA gyrase and topoisomerase IV modulate the topological state of DNA by passing an intact double helix through a transient double-stranded break that they generate in a separate segment [4, 8, 9]. By inhibiting DNA gyrase and topoisomerase IV the quinolones act by converting these targets into toxic enzymes that fragment the bacterial chromosome [9].

The first quinolone to be discovered was nalidixic acid in 1962, as a by-product of antimalarial research with choloroquine. Its use was limited to the treatment of urinary infections due to its narrow spectrum of antibacterial activity (only in front of several Gram negative bacteria), low serum and tissue levels, high tendency to produce resistance and toxicity issues [10, 11]. Fluorination of the quinolone nucleus at position 6 resulted in introduction of second, third and fourth generation agents with an increased antimicrobial spectrum of activity and better pharmacokinetic tissue-exposure characteristics [11]. Unfortunately, quinolone resistance in Gram-positive and Gram-negative bacteria has emerged and increased globally limiting the usefulness of quinolones in clinical practice [4] being the mutations in DNA gyrase and topoisomerase IV the most common cause of high-level quinolone resistance [9]. For instance, development and spread of quinolone resistance among clinical isolates have been greatest in *S. aureus*, particularly among methicillin-resistant strains, in which both selection by quinolone exposure and transmission of clonal strains in health-care settings have contributed to the high prevalence of quinolone resistance in this species [12]. In view of the patterns of resistance, there is consequently, a need for the development of newer agents with superior

activity against methicillin- and quinolone-resistant bacteria (e.g. methicillin- and quinolone-resistant staphylococci).

4.1.2 STRUCTURE AND HISTORY

The history of the development of the quinolones originated from nalidixic acid which was discovered accidentally in 1962 during the process of purification of choloroquine [13]. Nalidixic acid is a naphthyridone, not a quinolone: its ring structure is a 1,8-naphthyridine nucleus that contains two nitrogen atoms, unlike quinoline, which has a single nitrogen atom [14].Two years after its discovery the mechanism of action was defined as the inhibition of bacterial DNA-gyrase synthesis, by inhibiting the tertiary negative supercoiling of bacterial DNA, with a rapidly bactericidal effect [15, 16, 17, 18, 19]. It was much later, in 1990 that a homologue of DNA-gyrase, topoisomerase IV that had a potent decatenating activity was discovered and it was showed that topoisomerase IV, rather than DNA-gyrase, is responsible for decatenation of interlinked chromosomes [20]. The dual action against DNA-gyrase and topoisomerase IV has subsequently proved to be the same mechanism for all the antibacterial quinolones [18, 20]. In 1967, nalidixic acid was licensed for the treatment of urinary tract infections caused by the majority of Gram-negative bacteria, with the exception of *Pseudomonas aeruginosa*. Gram-positive organisms were usually resistant to the early quinolones. The clinical use of nalidixic acid, other than in the treatment of urinary infection, was therefore limited by its low serum concentrations and high minimum inhibitory concentrations. Early after marketing of nalidixic acid and its widespread clinical use, it was found that resistance developed rapidly in a number of organisms. This feature proved later to be a characteristic of the early quinolones. Subsequent derivations of nalidixic acid, such as pipemidic acid (the first piperazinyl quinolone), oxolinic acid and cinoxacin were discovered in the 1970s, and represented only marginal improvements over nalidixic acid [14].

Until the development of flumequine, the first monofluoroquinolone in 1976, none of the previous compounds offered any significant improvements over nalidixic acid. Flumequine was the first compound to be developed with a fluor-group at position 6 (flouroquinolone), and gave the first indications that modifications of the basic chemical structure could improve the Gram-positive activity [21]. In 1978 norfloxacin, a 6-fluorinated quinolone with a piperazinyl side-chain at position 7, was developed. In 1986 norfloxacin was licensed in the United States for its use in genitourinary infections. Norfloxacin had a longer and improved Gram-negative activity in relation to earlier compounds [22]. The first trifluorinated quinolone fleroxacin, entered the third decade of development and use. Fleroxacin was distinguished from its predecessors by its excellent bioavailability, high concentrations in the plasma and other body fluids, good tissue penetration and a long half-life (10–12 h), allowing for once-a-day administration. However, the incidence of side effects reported with fleroxacin, including severe phototoxic reactions, limited the clinical utility of this drug [23]. Between 1979 and 1982 a number of fluoroquinolones were

patented, including ciprofloxacin in 1981, which is still in widespread clinical use today. These compounds were much more active than earlier derivatives against *Enterobacteriaceae*, *P. aeruginosa* and many Gram-positive cocci. The fluoroquinolones developed in the third decade, such as ciprofloxacin and ofloxacin, are considered as having only moderate activity against pneumococcus, although the clinical outcomes have been somewhat better than those predicted by laboratory MICs. Compounds of the fourth decade of discovery improved (e.g. trovafloxacin, gatifloxacin, grepafloxacin) are active against primary pathogens that cause typical respiratory disease, e.g. *S. pneumoniae, H. influenzae and M. catarrhalis*) [14].

Over the past years fluoroquinolone research has been aimed at generally improving activity against Gram-positive pathogens, specially, against pneumococci, and improved activity against anaerobes, whilst retaining the activity against Gram-negative organisms. Further attempts to improve the pharmacological and antimicrobial properties of quinolones have led to the development of a new group of 'novel', 'third generation' fluoroquinolones used mainly for respiratory tract infections. These compounds are characterized by enhanced activity against Gram-positive cocci as well as many intracellular pathogens whilst retaining excellent activity against Gram-negative organisms, and with some activity against anaerobes. Examples of these agents are: trovafloxacin, moxifloxacin, gatifloxacin, gemifloxacin and grepafloxacin, are active against which have activity over all the primary pathogens that cause typical respiratory disease, e.g. S. pneumoniae, H. influenzae and M. catarrhalis [24, 25, 26].

Although currently more than 10,000 compounds have been already synthesized in the world, only two percent of them were developed and tested in clinical studies and approximately twenty of them have been successfully launched into the market [27].

The table below summarizes the chronology of development and use of main quinolones [14].

Table 1. Chronology of development and use of main quinolones

Decade	Compound
First decade (1960s)	Nalidixic acid
Second decade (1970s)	Flumequine Pipemidoc acid Oxolinic acid Cinoxacin Norfloxacin
Third decade (1980s)	Ciprofloxacin Ofloxacin

Decade	Compound
Fourth decade (1990s)	Temfloxacin* Sparfloxacin* Grepafloxacin* Trovafloxacin* Levofloxacin Gatifloxacin
Fifth decade (2000s)	Moxifloxacin
Sixth decade (2010s)	Gemifloxacin Garenoxacin
*Withdrawn	

4.1.3 CLASSIFICATION OF QUINOLONES

Different classifications have been proposed and/or used for the quinolone class of antibiotics.

The chemical classification is based on the chemical structure. According to this classification the quinolones are classified as: monocyclic, bicyclic, tricyclic, and tetracyclic and each of them can be sub-classified according of the presence of an atom of Fluor in position 6.

The biological classification is grouping the compounds by generations based on the timing of discovery, chemical structure, bacterial activity and clinical use. Some of the products are classified into one generation or another depending on the authors [27, 29, 30, 31, 32].

The table below summarizes the biological classification of quinolones together with the main indications of use [27, 29, 30, 31, 32].

Table 2. Biological classification of quinolones and main indications

Generation	Quinolone	Spectrum	Adm	Indications
First	Cinoxacin, Flumequine Nalidixic acid Oxolinic acid Pipemidic acid Piromidic acid Rosoxacin	Gram-negative bacteria: *E. coli, proteus, Klebsiella, Entrobacter, Citrobacter, Salmonella, Shigella.*	Oral	Non-complicated urinary tract infections
Second	Ciprofloxacin Enoxacin Fleroxacin Lomefloxacin Nadifloxacin Ofloxacin Norfloxacin Pefloxacin Rufloxacin	Same as first generation quinolones plus *P. aeruginose, N. gonorrhoeae, S. aureus, S. epidermidis, H. influenza, M. catarrhalis, mycobacteria* and atypical pathogens.	Oral, parenteral, topical	Non-complicated urinary tract infections, gastroenteritis, osteomyelitis, sexual transmitted diseases, respiratory tract infections, skin and skin structure infections.

Generation	Quinolone	Spectrum	Adm	Indications
Third	Balofloxacin Grepafloxacin Levofloxacin Pazufloxacin Sparfloxacin Temafloxacin Tosufloxacin	Similar to second plus Gram-positive bacteria such us S. *pyogenes, S. pneumonia,* and atypical pathogens.	Oral, parenteral	Non-complicated urinary tract infections, gastroenteritis, osteomyelitis, sexual transmitted diseases, respiratory tract infections, skin and skin structure infections, sepsis.
Fourth	Besifloxacin Clinafloxacin Garenoxacin Gemifloxacin Moxifloxacin · Gatifloxacin Sitafloxacin Trovafloxacin Alatrofloxacin Prulifloxacin	Similar to third plus anaerobic bacteria (*clostidium spp, bacteoides spp*).	Oral, parenteral	Gastroenteritis, osteomyelitis, sexual transmitted diseases, respiratory tract infections, skin and skin structure infections, abdominal infections, gynecological infections, sepsis.
Others	Ozenoxacin Nemonoxacin Delafloxacin Zabofloxacin	Similar to fourth with higher potency against Gram-positive, anaerobic, and atypical bacteria.	Oral, parenteral, topical	Respiratory tract infections, skin infections.

4.1.4 MECHANISM OF ACTION

The quinolone antibiotics act by inhibit to varying degrees the bacterial enzymes DNA gyrase and topoisomerase IV, which are responsible for introducing negative supercoils into DNA. This is an essential nucleic acid process that helps controlling the levels of DNA under- and over-winding, and removing knots and tangles from the bacterial chromosome. DNA gyrase and topoisomerase IV are therefore critical enzymes for the transcription and replication processes of bacterial DNA because as they have an important function of solving the accumulated superhelical tension ahead of polymerases and decatenating the two newly formed DNA strands [7].

DNA gyrase is responsible for introducing negative supercoils into DNA and for relieving topological stress arising from the translocation of transcription and replication complexes along DNA. It acts by wrapping DNA into a positive supercoil and then passing one region of duplex DNA through another via DNA breakage and rejoining. Keeping the DNA chromosome wound into loops facilitates the movement of replication forks. The reaction mechanism of topoisomerase IV is similar to that of gyrase but topoisomerase IV binds to DNA crossovers rather than wrapping DNA. Topoisomerase IV is primarily involved in decatenation, the unlinking of replicated daughter chromosomes [8]. Therefore, both DNA gyrase and topoisomerase IV modulate the topological state of DNA by passing an intact double helix through a transient double-stranded break that they generate in a separate segment [4, 8, 9]. By inhibiting DNA gyrase and topoisomerase IV the quinolones act by converting these targets into toxic enzymes that fragment the bacterial chromosome [9].

Quinolones act by binding to complexes that form between DNA and gyrase or topoisomerase IV inducing conformational change in the enzyme. The enzyme breaks the DNA and the quinolone prevents re-ligation of the broken DNA strands. The enzyme is

trapped on the DNA resulting in the formation of a quinolone–enzyme–DNA complex which inhibits DNA replication. This complex formation reversibly inhibits DNA and cell growth and is thought to be responsible for the bacteriostatic action of the quinolones. Lethal action is not reversible and is thought to be a separate event from complex formation. Higher concentrations of quinolone are needed to kill cells rather than to inhibit growth or form complexes and some quinolones inhibit growth better than others but are less effective at killing [8, 33]. Together with the formation of complexes, it is thought that the bactericidal action finally arises from the release of DNA ends from quinolone–gyrase–DNA complex [8, 34].

4.1.5 Resistance to quinolones

Mechanisms of quinolone resistance are generally grouped in three different types [4, 8, 9, 35]:

1. Chromosomal mutations altering the drug target enzymes to reduce drug binding.
2. Chromosomal mutations that increase expression of native efflux pumps that can transport quinolones to the outside of the bacterial cell,
3. Plasmid-acquired resistance genes producing either protection of target enzymes, drug modification, or drug efflux.

The cellular alterations associated with each mechanism are not mutually exclusive and can accumulate to create strains that exhibit very high levels of quinolone resistance.

Target-Mediated Quinolone Resistance

Quinolone resistance is usually associated with mutations in gyrase and/or topoisomerase IV and generally occur first in the GyrA subunit of DNA gyrase in Gram-negative bacteria or in the ParC subunit of topoisomerase IV in Gram-positive bacteria [4, 36]. These mutations associated with resistance to quinolones occur most often in a region known as the quinolone resistance determining region (QRDR) which encompasses amino acids 51 to 106 in GyrA and 23 to 176 in ParC, with positions 83 and 87 most common in GyrA and positions 80 and 84 most common in ParC [36, 37, 38]. These substitutions are thought to result in a reduced affinity of gyrase or topoisomerase IV for quinolones [39, 40]. In *Staphylococcus aureus* or *Streptococcus pneumoniae*, the primary target mutations occur most frequently in ParC [41, 42]. In both Gram-negative and Gram-positive bacteria, combinations of mutations in both GyrA and ParC generally result in progressively higher levels of resistance. Less often mutations in GyrB and ParE have also contributed to resistance in clinical isolates [4, 9].

Chromosome-Mediated Quinolone Resistance

Bacteria have a number of different energy-dependent efflux systems in their cell membrane and envelope that can facilitate extrusion of potentially toxic agents, and many

of these efflux pumps have broad substrate profiles that can include quinolones [43]. The AcrAB-TolC system is the major pump contributing to quinolone resistance in *E. coli* [36]. Mutations in acrR, which represses acrAB, can increase pump expression [44]. In addition, mutations in marR, a repressor of marA, which activates acr-AB and tolC, also causes an increase of efflux [45]. marA also decreases the expression of OmpF, outer membrane porin protein [46]. Consequently, marR mutations have the dual effect of decreasing influx and increasing efflux of quinolones. acrAB expression is also induced by exposure to salicylates and bile salts, and AcrAB confers relative resistance to bile salts, thereby facilitating the ability of *E. coli* to live the intestinal tract [47]. Efflux pumps that include quinolones among their substrates have also been associated with resistance in a number of other Gram-negative bacteria, being most extensively studied in *Pseudomonas aeruginosa* [48]. There are at least five known efflux pumps (MexAB-OprM, MexCDOpr-J, Mex- EF-OprN, MexXY-OprM, and MexVW-OprM) that have been shown to efflux quinolones in *P. aeruginosa*. In *S. aureus*, quinolone resistance has been associated with increased expression of NorA, NorB, and NorC pumps with both norA and norB overexpression regularly found in resistant clinical isolates [49, 50]. Efflux also contributes to quinolone resistance in S. pneumoniae and mycobacteria [4].

Plasmid-Mediated Quinolone Resistance

Plasmid-mediated quinolone resistance (PMQR) unlike target-mediated resistance, which is transmitted vertically from generation to generation, can be transmitted horizontally (through bacterial conjugation) as well as vertically. Plasmids that confer quinolone resistance typically carry additional genes that cause resistance to other drug classes [9]. PMQR was discovered in a clinical isolate of *Klebsiella pneumoniae* that was able to transfer quinolone resistance to Gram-negative bacteria [51]. The responsible gene for PMQR was named qnr (later named qnrA). The Qnr protein was shown to bind and protect DNA gyrase and topoisomerase IV from inhibition by ciprofloxacin [36]. Qnr provides only low-level resistance to quinolones, but its presence can facilitate the selection of additional resistance mutations [36]. It was then soon discovered in a growing number of organisms broadly distributed geographically [52, 53, 54, 55]. qnrA was subsequently followed by discovery of plasmid-mediated qnrS, nrB, qnrC, and qnrD [56, 57, 58, 59]. More recently other PMQR mechanisms have been identified. One is aac (6')-Ib-cr, which is a variant of aac (6')-Ib, encoding an aminoglycoside acetyltransferase [60]. aac (6')-Ib-cr confers low-level ciprofloxacin resistance by acetylation of ciprofloxacin at the amino nitrogen on its piperazinyl substituent and has also been associated with other PMQR genes including diverse qnr genes and beta-lactamase genes [61]. The other PMQR mechanism is plasmid-mediated quinolone efflux. Two plasmid-mediated quinolone transporters have also been identified: OqxAB [62] and QepA [63].

4.1.6 THERAPEUTIC INDICATIONS

Different infectious diseases are successfully treated with quinolones administered orally or intravenously. Clinical efficacy has been demonstrated for respiratory tract infections, including acute bacterial exacerbations of chronic bronchitis, community-acquired pneumonia, nosocomial pneumonia, and bacterial sinusitis. Quinolones also have documented clinical efficacy for the treatment of uncomplicated (and some complicated) urinary tract infections, bacterial prostatitis, skin and other soft-tissue infections, bone and joint infections, gastrointestinal infections caused by toxigenic *E. coli* or *Salmonella* species (including typhoid and paratyphoid fevers and the chronic *Salmonella* carrier state), and infection with *Shigella, Campylobacter, Aeromonas, Vibrio* species and *Plesiomonas shigelloides*. The quinolones have also been effective in treating sexually transmitted diseases, such as gonococcal and chlamydial infections, chancroid, and pelvic infections. Some quinolones have been very useful in treating immunocompromised patients with febrile neutropenia [64]. The quinolones have been widely used to treat community acquired as well as hospital infections [65].

Not all fluoroquinolones have been approved for use in the treatment of all of the aforementioned infections. The approved indications vary depending on country and on the specific product. The interchange between fluoroquinolones, especially for unapproved indications, is not recommended [64].

Some of the most frequently prescribed fluoroquinolones are ciprofloxacin, levofloxacin, gatifloxacin, gemifloxacin, and moxifloxacin [66].

The main approved indications for the most commonly used quinolones are indicated in the table below [64, 65].

Table 3. Main approved indications for the most commonly used quinolones

Agent	Main approved indications*
Ciprofloxacin	Acute uncomplicated cystitis in females (oral use only) Urinary tract infections Chronic bacterial prostatitis Uncomplicated cervical and urethral gonorrhea Skin and skin-structure infections Bone and joint infections Infectious diarrhea (oral use only) Typhoid fever (oral use only) Complicated intra-abdominal infections, in combination with metronidazole Acute sinusitis Lower respiratory tract infections Nosocomial pneumonia (iv use only) Empirical therapy for patients with febrile neutropenia, in combination with piperacillin sodium (iv use only)

Agent	Main approved indications*
	Inhalational anthrax (after exposure) Complicated urinary tract infections and pyelonephritis in pediatric patients (1–17 years old)
Levofloxacin	Complicated urinary tract infections and pyelonephritis in pediatric patients (1–17 years old) Levofloxacin Uncomplicated urinary tract infections (mild to moderate) Complicated urinary tract infections (mild to moderate) Acute pyelonephritis (mild to moderate) Chronic bacterial prostatitis Uncomplicated skin and skin-structure infections (mild to moderate) Complicated skin and skin-structure infections Acute maxillary sinusitis Acute bacterial exacerbation of chronic bronchitis Community-acquired pneumonia Nosocomial pneumonia
Moxifloxacin	Acute bacterial sinusitis Acute bacterial exacerbation of chronic bronchitis Community-acquired pneumonia Uncomplicated skin and skin-structure infections
Gatifloxacin	Uncomplicated urinary tract infections Complicated urinary tract infections Pyelonephritis Uncomplicated urethral and cervical gonorrhea Acute uncomplicated gonococcal rectal infections in women Uncomplicated skin and skin-structure infections Acute sinusitis Acute bacterial exacerbation of chronic bronchitis Community-acquired pneumonia
Gemifloxacin	Acute bacterial exacerbation of chronic bronchitis Community-acquired pneumonia (mild to moderate)
*Indications may vary depending on the countries	

4.1.7 USE OF QUINOLONE IN PEDIATRIC POPULATION

The first quinolone, nalidixic acid, developed in the 1960s was used off-label in pediatric patients without restriction. Consequent to their broad spectrum of antimicrobial (including anti-mycobacterial) effect and perceived excellent safety profile, there was considerable hope and expectation that this class of antibiotics would find an important place in pediatric therapeutics. However, reports of quinolone-associated injury in weight bearing joints of juvenile animals resulted not only in an apparent contraindication to their use in human infants and children but also, completely prevented their formal development by pharmaceutical companies for use in pediatrics. Although this situation resulted from a genuine concern for safety raised preclinical experimental findings, it served initially to remove a potentially useful class of antimicrobial agents from their pediatric use [67].

Despite the concerns associated with fluoroquinolone use in children, the favorable characteristics of this drug class (e.g., excellent oral bioavailability and tissue penetration, broad antimicrobial spectrum, well characterized and predictable concentration-effect relationships, relative low incidence of development of microbial resistance) resulted in their increasing use in infants and children; initially as secondary or tertiary antimicrobial choices and later, as a potential first line modality of treatment recommended in standard pediatric compendia used throughout the world [67].

Recommendations from the American Academy of Pediatrics indicate that fluoroquinolones may be useful for treating infections in pediatric patients where no other (appropriate) oral agent is available, the infection is caused by a multidrug-resistant pathogen (such as Pseudomonas sp. and Mycobacterium strains) or prolonged oral treatment of Gram-negative bacterial infections (e.g., chronic osteomyelitis, exacerbations in patients with cystic fibrosis, infections in immunocompromised patients) is needed [67, 68, 69]. Therefore, quinolones appears to now have place in the pediatric therapeutic armamentarium.

Some of the uses of systemic administration of quinolones in pediatric population are the following [70, 71, 72, 73, 74]:

- Broncho-pulmonary infections in cystic fibrosis with suspected/confirmed *Pseudomonas spp.* Infections.
- Immunocompromised patients.
- Neonatal sepsis/meningitis with multidrug-resistant Gram-negative bacilli.
- Severe enteric infections caused by Salmonella and *Shigella spp*.
- Complicated urinary tract infections with multidrug-resistant organisms.
- Chronic suppurative otitis media with *Pseudomonas spp*.
- Complicated acute otitis media failing to respond to initial antibiotic treatment.
- Inhalation anthrax (post-exposure prophylaxis and curative treatment).

Nadifloxacin a broad-spectrum, fluoroquinolone is approved in some countries for the topical use in acne vulgaris and skin infections [75]. ozenoxacin (a non-fluorinated quinolone) is currently in development as a 1% cream for the topical treatment of impetigo.

As mentioned above, the use of fluoroquinolones in pediatric patients has been limited due to arthropathy noticed in weight bearing joints of juvenile animals. Cartilage damage caused by quinolones (nalidixic, oxolinic and pipemidic acids) was initially reported in preclinical studies conducted in juvenile animals (e.g. beagle dogs 4–12 months of age) [76, 77]. Arthropathy has thereafter also been described in other animal species such as mice, dogs, rats and rabbits, and in in-vitro animal culture, and in-vitro human cell culture

[78, 79, 80, 81, 82, 83]. In these studies have documented cartilage injury in weight-bearing joints in juvenile animals; damage to the joint cartilage was proportional to the degree of exposure [79, 84, 85]. Each quinolone may demonstrate a different potential to cause cartilage toxicity [86]; however, given a sufficiently high exposure, cartilage changes would occur in all animal models with all quinolones [84]. Although initial reports focused on articular cartilage toxicity, posterior studies suggested also the possibility of damage to epiphyseal plate cartilage injury [87]. Data for more recent studies data suggest that high quinolone concentrations quinolone in the cartilage form chelate complexes with divalent cations, particularly magnesium, that result in impairment of integrin function and cartilage matrix integrity in the weight-bearing joints, which the undergo chronic trauma during routine use [84, 88].

No definitive clinical published evidence supports the occurrence of sustained injury to developing bones or joints in children treated with available fluoroquinolone agents; published data of fluoroquinolone safety in children is available from retrospective studies, case-control series, and case reports. Some reports included children with cystic fibrosis, who can develop disease-related arthropathy. All these data provided conflicting reports regarding the safety of fluoroquinolones in children. however, FDA analysis of ciprofloxacin safety data, as well as post-treatment and 12-month follow-up safety data for levofloxacin, suggest the possibility of increased musculoskeletal adverse effects (such us arthralgia, abnormal joint and/or gait exam, accidental injury, leg pain, back pain, arthrosis, bone pain, joint disorder, pain, myalgia, arm pain, movement disorder) in children who receive fluoroquinolones compared with agents of other classes [84, 89].

Achilles tendon rupture (which has been described in adults) is extremely rare in the pediatric population and up to date, there have been no reports of this rare complication in a pediatric population [84]. Other potential toxicities associated with quinolone antibiotics do not occur commonly in children but include central nervous system (CNS) adverse effects (seizures, headaches, dizziness, lightheadedness, sleep disorders), peripheral neuropathy, hypersensitivity reactions, photosensitivity and other rashes, disorders of glucose homeostasis (hypoglycemia and hyperglycemia), prolongation of QT interval, and hepatic dysfunction) [84].

In general, restrictive use of systemic quinolones is still recommended in pediatric population, because of a slight risk of quinolone-induced arthropathy cannot be excluded; and because concerns about the rapid spread of resistant pneumococci. Treatment with a systemic quinolone in pediatric population should be restricted to the indications commented above and when the patient suffers from a life-threatening or severe infection and, second, other antibiotics cannot be used because of patient allergy or tolerability or because the pathogen is resistant to other anti-infective drugs [88].

4.1.8 ADVERSE EFFECTS OF QUINOLONES

Initially originated from anti-malarial drugs, the modern quinolones have been developed evolved via nuclear and side-chain modification from 1,8-naphthyridine (first generation) molecules to compounds characterized by ever increasing the spectrum of activity and potency, together with trends to longer elimination half-lives which allow once daily dosing. The individual compounds also have somewhat differing class and specific adverse drug reaction (ADR) profiles [90, 91].

Some of the ADR associated with the different quinolones are summarized as follows [91, 92]:

- Several compounds of the first generation, notably pipemidic acid, caused significant CNS problems and small piperazine-like side-chains at the 7-position are recognized to be associated with a higher incidence of CNS ADRs.
- Some compounds were withdrawn from the market or discarded during development due to adverse reactions (temafloxacin due to haemolytic uraemic syndrome, grepafloxacin due to cardiac toxicity and sparfloxacin due to cardiotoxicity and phototoxicity. The ADR described are considered class ADR such us: CNS effects, gastrointestinal, skin rashes and allergic reactions, phototoxicity (usually <2, tendinitis (usually minor) renal and hepatic syndromes; cytochrome P450 interaction (theophylline, caffeine).
- Some compounds have a good safety and tolerability profile. Specific ADRs have been described with trovafloxacin, e.g. higher incidence of CNS effects, hepatic, allergic reactions and pancreatitis; also specific effects with clinafloxacin, including hypoglycaemia and increased incidence of severity of phototoxicity.
- Some compounds of the last generation quinolones have a favorable ADR profile with low CNS ADR rate, with no or low phototoxic potential. QT-prolongation has been described.
- Although the newer compounds have a favorable safety profile, there are certain major class effects that vary in incidence and severity amongst the quinolones, often in association with known structural configurations such as cardiac effects, CNS disturbance, and tendinitis. Phototoxicity has apparently almost completely designed out of modern compounds (although latterly affecting clinafloxacin) [91].

An example of the safety and tolerability profile of quinolones can be taken from ciprofloxacin. The ADRs derived from clinical studies and post-marketing surveillance with ciprofloxacin (with oral and intravenous administrations) sorted by MedDRA system organ class (SOC) and by categories of frequency are listed in the table below [74].

Table 4. ADRs derived from clinical studies and post-marketing surveillance with ciprofloxacin (with oral and intravenous administrations)

SOC	Common ≥ 1/100 to < 1/10	Uncommon ≥ 1/1,000 to < 1/100	Rare ≥ 1/10,000 to < 1/1,000	Very Rare < 1/10,000	Frequency not known
Infections and Infestations		Mycotic super-infections	Antibiotic associated colitis (very rarely with possible fatal outcome)		
Blood and Lymphatic System Disorders		Eosinophilia	Leukopenia Anaemia Neutropenia Leukocytosis Thrombocytopenia Thrombocytaemia	Haemolytic anemia Agranulocytosis Pancytopenia (life-threatening) Bone marrow depression (life-threatening)	
Immune System Disorders			Allergic reaction Allergic edema / angioedema	Anaphylactic reaction Anaphylactic shock (life-threatening) Serum sickness-like reaction	
Metabolism and Nutrition Disorders		Decreased appetite	Hyperglycemia Hypoglycaemia		
Psychiatric Disorders		Psychomotor hyperactivity / agitation	Confusion and disorientation Anxiety reaction Abnormal dreams Depression (potentially culminating in suicidal ideations/thoughts or suicide attempts and completed suicide) Hallucinations	Psychotic reactions (potentially culminating in suicidal ideations/thoughts or suicide attempts and completed suicide)	
Nervous System Disorders		Headache Dizziness Sleep disorders Taste disorders	Par- and Dysesthesia Hypoesthesia Tremor Seizures (including status epilepticus) Vertigo	Migraine Disturbed coordination Gait disturbance Olfactory nerve disorders Intracranial hypertension and pseudotumor cerebri)	Peripheral neuropathy and polyneuropathy
Eye Disorders			Visual disturbances (e.g. diplopia)	Visual color distortions	
Ear and Labyrinth Disorders			Tinnitus Hearing loss / Hearing impaired		

SOC	Common ≥ 1/100 to < 1/10	Uncommon ≥ 1/1,000 to < 1/100	Rare ≥ 1/10,000 to < 1/1,000	Very Rare < 1/10,000	Frequency not known
Cardiac Disorders			Tachycardia		Ventricular arrhythmia and torsades de pointes (reported predominantly in patients with risk factors for QT prolongation), ECG QT prolonged
Vascular Disorders			Vasodilatation Hypotension Syncope	Vasculitis	
Respiratory, Thoracic and Mediastinal Disorders			Dyspnea (including asthmatic condition)		
Gastro-intestinal Disorders	Nausea Diarrhea	Vomiting Gastro-intestinal and abdominal pains Dyspepsia Flatulence		Pancreatitis	
Hepatobiliary Disorders		Increase in transaminases Increased bilirubin	Hepatic impairment Cholestatic icterus Hepatitis	Liver necrosis (very rarely progressing to life-threatening hepatic failure)	
Skin and Subcutaneous Tissue Disorders		Rash Pruritus Urticaria	Photosensitivity reactions	Petechiae Erythema multiforme Erythema nodosum Stevens-Johnson syndrome (potentially life-threatening) Toxic epidermal necrolysis (potentially life-threatening)	Acute generalised exanthematous pustulosis (AGEP)
Musculo-skeletal and Connective Tissue Disorders		Musculo-skeletal pain (e.g. extremity pain, back pain, chest pain) Arthralgia	Myalgia Arthritis Increased muscle tone and cramping	Muscular weakness Tendinitis Tendon rupture (predominantly Achilles tendon) Exacerbation of symptoms of myasthenia gravis	

SOC	Common ≥ 1/100 to < 1/10	Uncommon ≥ 1/1,000 to < 1/100	Rare ≥ 1/10,000 to < 1/1,000	Very Rare < 1/10,000	Frequency not known
Renal and Urinary Disorders		Renal impairment	Renal failure Haematuria Crystalluria Tubulointerstitial nephritis		
General Disorders and Administration Site Conditions		Asthenia Fever	Edema Sweating (hyperhidrosis)		
Investigations		Increase in blood alkaline phosphatase	Increased amylase		International normalized ratio increased (in patients treated with Vitamin K antagonists)

Some of the most relevant ADR associated with quinolones in general are described below.

Cardiac effects

Cardiovascular effects, particularly prolongation of the QT interval corrected for heart rate (QTc interval), has been reported with quinolone therapy [93]. The effect has been described for sparfloxacin [94, 95] and also with grepafloxacin which was withdrawn due to severe cardiac events [93]. Although, no specific structural modifications have been associated with the cardiovascular effects, the only possible specific structural modifications that may be associated with the increased incidence of serious cardiovascular events associated with grepafloxacin and sparfloxacin therapy are a methyl or amino moiety at the C-5 position (grepafloxacin and sparfloxacin, respectively) [91]. Reports have also been recorded as well for other quinolones such as moxifloxacin, levofloxacin, gatifloxacin, gemifloxacin and grepafloxacin [90, 91, 96, 97]. The prolongation of the QT interval is associated with a risk of potentially fatal cardiac arrhythmias (e.g., torsades de pointes, sudden death), due to repolarization disturbances [93, 98].

In-vitro studies have shown that the most common cause of drug-induced QT prolongation is block of the human ether a-go-go–related gene (HERG)-encoded delayed rectifier potassium current, IKr [98, 99, 100, 101].

Although the risk of arrhythmia is small (<1/1 million treated cases) for the contemporary fluoroquinolones [91, 93], it is appropriate to recognize patients already at increased risk (e.g. elderly females with electrolyte disturbance or significant cardiac disease, patients with a history of arrhythmia and/or who are receiving anti-arrhythmic or other agents known significantly to prolong the QT interval, like antihistamines and cisapride) and use these agents with caution in this population [91].

Hepatic toxicity

Increase of liver enzymes has been that recover after treatment withdrawal has been described with the use of quinolones. A low percentage of patients can present hepatitis, hepatic necrosis, and liver failure [97]. Hepatic-related injury involving eosinophilic infiltration, hepatocellular vacuolar degeneration, and necrosis were reported with trovafloxacin [102].

The pathophysiology of the adverse hepatic events of trovafloxacin is not known although it has been suggested that the addition of a 2,4-difluorophenyl moiety at C-1 may be the cause of the toxic effects of this agent. [90, 102, 103, 104].

Hemolytic uremic syndrome

Temafloxacin has been associated with a relatively uncommon immune-related toxicity syndrome characterized by hemolysis, renal failure, and thrombocytopenia (temafloxacin syndrome) which occurred at an estimated incidence of 1 case/5,000 prescriptions [105].

CNS effects

CNS symptoms following administration of quinolones have been reported at an overall incidence of 1%-2% of cases. A 12.2% incidence of CNS ADR has been estimated from spontaneous adverse event reports [106]. The more commonly reported symptoms have included dizziness, headache, and somnolence [90, 91, 97, 102]. Other, less commonly reported, CNS ADRs have included agitation, delirium, confusion, acute organic psychosis, and abnormal vision. Seizures are a rare ADR and usually involve a susceptible population with underlying CNS disorders, such as epilepsy, cerebral trauma, or anoxia [102, 103].

Overall, quinolones with the potential for causing CNS-related adverse events may be listed, from higher potential to lower potential, as follows: fleroxacin, trovafloxacin, grepafloxacin, norfloxacin, sparfloxacin, ciprofloxacin, enoxacin, ofloxacin, pefloxacin, gatifloxacin, gemifloxacin, levofloxacin, and moxifloxacin [97, 102].

Although the mechanism of the CNS effects is unclear, one hypothesis suggests that drug interactions with the gamma-aminobutyric acid receptor (GABAa), could explain CNS-stimulating effects. The R7 side chain substituent, particularly unsubstituted piperazinyl and pyrrolidinyl moieties appears to have affinity for the GABA receptor. Therefore, agents with an unsubstituted piperazinyl ring (ciprofloxacin, enoxacin and norfloxacin) demonstrate high-affinity binding to GABAa and interfere with GABA binding to its receptor [107]. It has also suggested that fluoroquinolones can also induce excitatory effects through direct activation of N-methyl-Daspartate (NMDA) and adenosine-receptor mechanisms [90].

Gastrointestinal effects

Gastrointestinal ADR have included nausea, anorexia, vomiting, abdominal pain, diarrhea, and taste disturbance with a general incidence of 2%–20%. The agents with the highest to lowest associated probability are as follows: fleroxacin, grepafloxacin, trovafloxacin, sparfloxacin, pefloxacin, ciprofloxacin, gatifloxacin, gemifloxacin, levofloxacin, moxifloxacin, norfloxacin, enoxacin, and ofloxacin [97, 102]. A 9.1% incidence of CNS ADR has been estimated from spontaneous adverse event reports [106]. *C. difficile* associated diarrhea has been described with quinolones; specially , with fourth generation quinolones [100, 108].

Musculoskeletal effects

A rare complication associated with quinolone antibiotic agents tendinitis and tendon rupture, with special predilection for the Achilles tendon (often bilateral) is estimated to occur at a rate of 15 to 20 per 100.000 treated patients in the adult population and has been associated with different risk factors such as advanced age, along with antecedent steroid therapy and a particular subset of underlying diseases, including hypercholesterolemia, gout, rheumatoid arthritis, end-stage renal disease/dialysis, and renal transplantation, have been identified [84, 109]. Such events are bilateral in 50% of cases. Predisposing factors can include corticosteroid therapy, renal disease, hemodialysis and transplantation [110]. Usually symptoms resolve within weeks, but in a small proportion of patients, they may persist for months [91]. This effect was originally observed with pefloxacin, but has subsequently been reported with almost all class members [111, 112]. Significant differences have been observed in the frequencies between agents, with levofloxacin and pefloxacin being associated with more reports than were ciprofloxacin, enoxacin, moxifloxacin, and rufloxacin. According to the reports from the US FDA MedWatch Spontaneous Adverse Events reporting system, the frequencies of tendon disorders are between 0.05 (ciprofloxacin) and 0.6 (levofloxacin) per 100 000 treatments [91].

The mechanism of tendon injury seem to be similar to that of quinolone-related arthropathies; in animal models, after treatment with ofloxacin, magnesium-deficient animals show a more pronounced injury to tendons [102; 113].

Photosensitivity and phototoxicity

The chemically-related quinolone family is associated with photosensitivity and phototoxicity reactions [90, 91, 97, 102, 103]. mostly related to halogenation (chlorine, fluorine) of position 8 in concert with the fluorination of position 6 i.e., the so-called double-halogenated quinolones) which has demonstrated a significant phototoxic potential. The photo-degradation of the fluoroquinolone molecule by UVA light (around 350 to 360 nm) generates monovalent oxygen radicals and other free radicals that attack the lipid membranes, initiating inflammatory processes and eventually resulting in DNA damage [102, 103, 114, 115, 116, 117].

The overall phototoxic-potential ranking of certain quinolones, from highest phototoxicity to lowest has been described as follows: lomefloxacin, fleroxacin, clinafloxacin, sparfloxacin, enoxacin, pefloxacin, ciprofloxacin, grepafloxacin, gemifloxacin, levofloxacin, norfloxacin, ofloxacin, trovafloxacin, gatifloxacin, and moxifloxacin [97, 102].

Glucose homeostasis

The quinolones, as a class, have demonstrated the capacity to close K^+-ATP channels in the pancreatic β-ell, resulting in release of insulin and subsequent hypoglycemia [102, 118]. The insulinotropic effects have been also described for quinine which is related by the chemical structure (quinoline rings) to the quinolone antibiotics. Hyperglycemia has also been reported for quinolones although the mechanism is poorly understood [102].

Anaphylaxis

Anaphylaxis reactions (type I, IgE-mediated reactions occurring within 1 h of administration) such us urticaria, angioedema, and anaphylactic shock have been reported in relation to quinolone use [102]. These reactions occur less frequently with quinolones use than with the use of other antimicrobial classes, such as the b-lactams [119] and have been associated with quinolone-specific IgE, and a substantial cross-reactivity seem to exist among various quinolones [120].

Immune-related idiosyncratic reactions

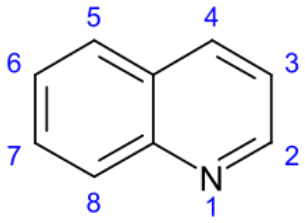
Some relatively uncommon immune-related toxicities have been associated to quinolone use, including hemolytic-uremic syndrome, hemolytic anemia, thrombocytopenia, leukopenia, acute interstitial nephritis, acute hepatitis, acute cholestatic jaundice, Stevens-Johnson and Lyell syndromes, fixed drug eruption, cutaneous vasculitis, macular-papular exanthema, acute pancreatitis, serum sickness–like disease, angioimmunoblastic lymphadenopathy, acute exathematous pustulosis, and eosinophilic meningitis [102, 119]. Some of these ADR such as hepatitis, pancreatitis, interstitial nephritis, hemolytic anemia, and hemolyticuremic syndrome, may be due to combined antibody and T cell interaction with the drug and host. Maculopapular exanthema has been associated with noncovalent interactions with T cell receptors and major histocompatibility complex [102].

Genetic toxicity

The inhibition of mammalian cellular topoisomerase II has been shown to correlate with in vitro cytotoxicity [102, 116]. Substitutions at the 1-, 7- and 8-positions have the greatest potential for cytotoxicity, with the effect being additive. However, disruption of the chromosome, or clastogenicity, usually occurs only at very high drug concentrations (300 to 10,000 times the normal dose level), and postmarketing surveillance studies have not found any carcinogenic potential linked to fluoroquinolone use [102].

As described above, some of the ADR have been related to the structure of the quinolone and the different radical substituents the molecule. The main structure-side-effect relationships of quinolone antibacterials is summarized in the table below [116]:

Table 5. Structure-side-effect relationships of quinolones

Position	Related side effect
1	Theophylline interaction and genetic toxicity
2	No side effects associated
3-4	Metal binding and chelation, interaction with antacids, milk, iron, divalent cations
5	Influence phototoxicity, genetic toxicity, cardiotoxicity
6	Phototoxicity (fluorination in 6 together with hologenation in position 8)
7	GABA binding, theophylline interaction
8	Phototoxicity
	

4.1.9 NEW QUINOLONES UNDER DEVELOPMENT

New antimicrobial agents from the quinolone group are in development for different indications.

The table below presents the new antimicrobial agents from the quinolone group and hybrid compounds (with the quinolone structure) that are in development [121].

Table 6. New quinolones under development

Class	Drug Name	Status	Main activity	Indication	Route
Non-florinated quinolones	Nemonoxacin (TG-873870)	Phase II	*S. pneumonia, S. aureus, MRSA, Vancomycin resistant, Enterococci, Nocardi spp.*	CAP	Oral
	Ozenoxacin	Phase III	*S. aureus, MRSA, S. epidermidis, S. pyogenes, P. acnes.*	Impetigo	Topical
	KRP-AMI1977X	Phase III	Gram-positive bacteria, *MRSA*	NA	Oral
Fluorinated quinolones	Zabofloxacin	Phase III	*S. pyogenes, E. faecalis, S. pneumoniae, H. influenzae, M. catarrhalis*	ABSSI, Exacerbation of COPD	Oral
	Finafloxacin (BAY353377)	Phase II	*E. coli, K. pneumoniae, S. aureus, MRSA, P. aeruginosa in comparison H. pylori*	Erradication *H. pylori* infections	Oral
	Delafloxacin	Phase III	*S. aureus, S. pneumonia, S. epidermidis*	ABSSSI, MRSA	Oral
	Acorafloxacin, JNJ-32729463	Phase II	*S. aureus, MRSA*	ABSSSI, MRSA	Oral

	WCK771	Phase II	*Methicillin and vancomycin strains of S. aureus, staphylococci resistant to currently aavailable quinolones, and anaerobic bacteria*	Different infections caused by susceptible bacteria	Oral
	KPI-10 (WQ3813)	Phase I	*S. aureus, S.epidermidis, S. pneumoniae, S. pyogenes, S. Agalactia, E. faecalis*	NA	Oral
Hybrid compounds (containing the quinolone structure)	Cadazolid (ACT-179811)	Phase III	*C. difficile*	*Clostridium difficile* associated diarrhea	Oral
	CBR2092	Phase I	*Staphyloccocus*	*Staphylococcal* infections	Oral

4.2 ACUTE BACTERIAL SKIN AND SKIN STRUCTURE INFECTIONS

Acute bacterial skin and skin structure infections (ABSSSI) previously referred to as uncomplicated and complicated skin and skin structure infections wide spectrum of disease. The disease can range from mild to severe, and includes [121, 122, 123]:

- Impetigo.
- Abscesses.
- Erysipelas.
- Folliculitis and furunculosis.
- Cellulitis.
- Carbuncles.
- Necrotizing fasciitis.
- Secondarily infected traumatic lesions.
- Secondarily infected dermatoses.
- Other soft tissue infections.

Soft tissue refers to tissues that connect, support, or surround other structures and organs of the body that are not bone (e.g. muscle, tendons, fat, and blood vessels). The mechanism of such infections varies and may result secondary to minor or major abrasions, wounds, trauma, animal or human bites, or surgical site infections, etc [116].

ABSSSI are typically caused by Gram-positive pathogens, including *S. aureus* and ß-hemolytic streptococci (*S. pyogenes*). However certain Gram-negative and anaerobic bacteria are also found in polymicrobial infections. Over the past decade, widespread emergence of community-acquired methicillin-resistant *S. aureus* (CA-MRSA) has been reported. Previously, MRSA infections were limited to hospital-acquired (or other

nosocomial sources) infections. Most of the ABSSSIs can be treated successfully in the outpatient setting but complicated infections or those due to resistant organisms usually require systemic (oral or intravenous) treatment and/or hospitalization [116]. Various highly virulent community-associated MRSA clones have been identified worldwide as responsible agents of skin and soft-tissue infections and necrotizing pneumonia in otherwise healthy adults and children [124]. An increasing prevalence of asymptomatic colonization by MRSA among children and adults in the community has been described in the western world [125]. For instance, MRSA has been identified as the most common cause of skin and soft-tissue infections among patients presenting to emergency departments in U.S. Therefore, it has been proposed that when antimicrobial therapy is indicated for the treatment of skin and soft-tissue infections, clinicians should consider obtaining cultures and modifying empirical therapy to provide MRSA coverage [126].

Uncomplicated skin and skin structure infections (SSSIs) can occur in the various layers of the skin. The different type of ABSSSIs can be more or less invasive affecting different layers of the skin.

The table below show the different layers of the skin affected in the most common ABSSSIs [127].

Table 7. Layers of the skin affected in ABSSSIs

Layer	Infection
Epidermis	Impetigo, secondarily infected atopic dermatitis, erysipelas, furunculosis
Dermis	Secondarily infected atopic dermatitis, Cellulitis, cutaneous abscess, erysipelas, folliculitis, furunculosis
Hypodermis	Cellulitis, cutaneous abscess

Therapeutic options for uncomplicated ABSSSIs include incision and drainage in combination with antimicrobial therapy, which may be oral, topical or occasionally parenteral. Because the effectiveness of the current oral options, such as beta-lactams and other classes, is being eroded due to acquired resistance, older, untested agents such as trimethoprim-sulfamethoxazole or clindamycin are often being used. Thus, topical approaches may offer effective, localized, well-tolerated alternatives to the systemic regimen [127, 128]. Some of the most commonly prescribed antibiotics for uncomplicated ABSSSIs administered either topically, orally, or intravenously are: mupirocin, retapamulin, cephalexin, amoxicillin, amoxicillin/clavulanic acid, doxycicline, minocycline, trimethoprim/sulfamethoxazol, metronidazole, different fluoroquinolones, dicloxacilin, vancomycin, piperacillin-tzobactam, ceftriaxone, cefazolin, ceftibuten, and cefdinir [128].

4.3 IMPETIGO

Impetigo is a contagious superficial bacterial skin infection most common in children which can occur as a primary infection or be secondary to other skin disorders which disrupt the skin barrier, such as atopic dermatitis or scabies. Impetigo may occur in two clinical forms: bullous (or impetigo contagiosa) and non-bullous, the latter being the most prevalent one occurring in more than 70% of cases. Nearly all cases of impetigo are caused by *Staphylococcus aureus* and *Streptococcus pyogenes* alone or in combination. *S. aureus* and *S. pyogenes* can cause different skin infections, especially ABSSSI where they represent up to 52% of all cases and in the specific disease of impetigo nearly all the cases [129]. Non-bullous impetigo is the most common form of impetigo and is caused by *S. aureus* or *S. pyogenes* and bullous impetigo is invariably caused by toxin-producing S aureus. Impetigo is the third most common skin disease in children (after dermatitis and viral warts), with a peak incidence at 2-6 years of age [130, 131, 132]. Lesions are highly contagious and can spread rapidly by direct contact, through a family, nursery, or class [130, 133]. The condition is more common in children with atopic dermatitis, in those living in tropical climates, and in conditions of overcrowding and poor hygiene. Nasal carriage of organisms may predispose to recurrent infection in an individual. The lesions begin as vesicles or pustules that rapidly evolve into gold-crusted plaques (often 2 cm in Diameter) that can usually affect the face and extremities and heal without scarring. Constitutional symptoms are normally are absent. Satellite lesions may occur due to autoinoculation. Bullous impetigo is characterized by flaccid, fluid filled vesicles and blisters (or bullae). These lesions are normally painful, spread rapidly, and can produce systemic symptoms. Lesions are often multiple, particularly around the oronasal orifices, and grouped in body folds [130]. The annual global disease burden is estimated to be 111 million cases [134, 135].

There is uncertainty in relation to the optimal treatment of impetigo. The management options for impetigo can include the following:

1. No pharmacological treatment, waiting for natural resolution, hygiene measures.
2. Topical disinfectants (such as saline, hexachlorophene, povidone iodine, and chlorhexidine).
3. Topical antibiotics (such as neomycin, bacitracin, polymyxin B, gentamycin, fusidic acid, mupirocin, retapamulin, or topical steroid/antibiotic combination).
4. Systemic antibiotics (such as penicillin, (flu)cloxacillin, amoxicillin/clavulanic acid, erythromycin, and cephalexin).

The aim of treatment includes resolving the soreness caused by lesions and the disease's unsightly appearance (especially on the face), as well as preventing recurrence and spread to other people. An ideal treatment should be effective, cheap, easy to use, and accepted by

people. It should be free from side-effects, and it should not contribute to bacterial resistance. For this reason, it is considered that antibiotics should not have an unnecessarily broad spectrum [136, 137, 138] and if a topical antibiotic is used, it should, preferably, not be one which may be also needed for systemic use [136, 138, 139].

Waiting for natural resolution could be an acceptable approach if the natural history were known and benign because impetigo has been described to be self-limiting by many authors [140, 141]. However, there is no robust data available on the natural history of impetigo. The reported cure rates of placebo creams can vary from 8% to 42% at 7 to 10 days [136, 142, 143]. Topical cleansing used to be advised in the 1970s as an alternative for antibiotic treatment, but this has not been considered more effective than placebo [136, 144]. Topical cleansing alone is not usually considered in treatment Guidelines and treatment advice because the concern is preventing the spread of the infection to other children. Therefore, a choice has to be made between topical and systemic antibiotic treatment, although in some situations clinicians prescribe both topical and systemic antibiotics.

An advantage of the use of topical antibiotics is that the drug can be applied where it is needed, avoiding systemic side-effects such as gastrointestinal upset and compliance may be better compared to systemic administration [145]. However, the use of topical antibiotics can have some disadvantages such as the potential risk of developing bacterial resistance and sensitization, e.g. developing an allergic contact dermatitis to one of the constituents of the topical formulation [136, 138, 139]; especially with the older antibiotics, such as gentamycin, bacitracin, and neomycin [138]. Some formulations (e.g. including tetracycline) can cause staining of the skin and clothes. Staphylococcal resistance against penicillin and erythromycin is common [144] and bacterial resistance against the newer topical antibiotics, such as mupirocin ointment and fusidic acid ointment, is increasing [146, 147]; however, advantage is that mupirocin is never used systemically and fusidic acid is not often used systemically.

A recent Cochrane review evaluating 68 clinical trials with 5,578 participants, reporting on 50 different treatments for impetigo, including systemic and topical treatments (including new antibiotics such as retapamulin), topical disinfectant solutions and placebo [136], has found that there is no widely accepted standard therapy for impetigo.

The antibiotics that have been generally used in the treatment of bullous and non-bullous impetigo are the following [136]:

- Topical: mupirocin, fusidic acid, bacitracin, retapamulin, rifamycin, neomycin, polymyxin B, chlortetracycline, gentamycin.
- Oral: dicloxacillin, cephalexin, ampicillin, erythromycin, penicillin, cefdinir, minomycin, fosfomycin, amoxicillin/clavulanic acid, cefadroxil, flucoxacillin, cefditoren, cefuroxime, cefaclor, dicloxacillin, norfloxacin.

Most of the studies included in this evaluation did not contribute to clear answers about the vast choice of treatment options. Overall, topical antibiotics showed better cure rates than topical placebo. No differences were found between the two most studied topical antibiotics: mupirocin and fusidic acid. Topical mupirocin was superior to oral erythromycin. In most other comparisons, topical and oral antibiotics did not show significantly different cure rates, nor did most trials comparing oral antibiotics. Penicillin V was inferior to erythromycin and cloxacillin, and there is a lack of evidence to suggest that using disinfectant solutions improves impetigo. The reported number of side-effects was low. Oral antibiotic treatment caused more side-effects, especially gastrointestinal ones, than topical treatment [136].

The main details derived from this review are shown in the table below [136, 148]:

Table 8. Main results from impetigo intervention trials

Comparison	N patients	ARR	NNT
Topical antibiotics vs placebo	575	41.2%	2
Retapamulin vs placebo	213	33.5%	3
Topical antibiotics vs disinfectant	292	11.4%	9
Mupirocin vs fusidic acid	440	NS	NS
Mupirocin vs oral erythromycin	581	5.1%	20
Mupirocin vs dicloxacillin	53	NS	NS
Mupirocin vs ampicillin	13	NS	NS
Bacitracin vs oral erythromycin	30	NS	NS
Bacitracin vs oral erythromycin	30	NS	NS
Cephalexin vs bacitracin	19	56.7%	2
Erythromycin vs penicillin	79	22.4%	4
Cloxacillin vs penicillin	166	35.9%	3
ARR: absolute risk reduction (cure or improvement) **NNT:** number needed to treat (number of patients that need to be treated with test for one patient to benefit compared to control) **NS:** non-significant			

Based on the results of the different intervention trials in impetigo and taking into account the Strength of Recommendation Taxonomy (SORT) [149], the following clinical recommendations could be derived [136, 150]:

Table 9. SORT recommendations for practice for interventions for impetigo

Clinical recommendation	Evidence rating
Topical antibiotics are more effective than placebo and preferable to oral antibiotics for limited impetigo.	A
Oral penicillin should not be used for impetigo because it is less effective than other antibiotics.	B
Oral erythromycin and macrolides should not be used to treat impetigo because of emerging drug resistance.	B

There is insufficient evidence to recommend topical disinfectants for the treatment of impetigo.	B
There is insufficient evidence to recommend (or dismiss) popular herbal treatments for impetigo.	C
A = consistent, good-quality patient-oriented evidence B = inconsistent or limited-quality patient-oriented evidence C = consensus, disease-oriented evidence, usual practice, expert opinion, or case series.	

The Infectious Diseases Society of America (IDSA) recommends topical mupirocin as first-line therapy for limited impetigo, although resistance to the drug exists. For patients with numerous lesions or who fail to respond to topical treatment, the IDSA recommends oral antibiotics active against *S. pyogenes* and *S. aureus*. Recommended oral antibiotics include dicloxacillin, amoxicillin/clavulanate, cephalexin, erythromycin, and clindamycin [129].

Resistance to quinolones among Gram-positive cocci has emerged after these agents have become extensively used in clinical medicine [12]. Although different therapeutic alternatives are available for the treatment of impetigo, escalating numbers of bacteria causing SSSIs (including impetigo); have become resistant to leading topical antimicrobials used in human clinical practice to treat these kind infections. For instance, *S. aureus* has shown resistance rates to mupirocin (a topical antimicrobial agent) ranging from 1.3% in Latin America to 8.7% in Europe. The natural habitat of *S. aureus* as a common colonizer of skin and nares probably contribute in part to opportunities for *S. aureus* exposure to fluoroquinolones and other antibiotics used for conventional treatment indications. Resistance could be been promoted by the presence of the drug concentrations delivered to cutaneous and mucosal surfaces harboring colonizing *S. aureus* [12]. Mupirocin resistance increases in coagulase negative *Staphylococci*, ranging from 12.7% in Europe to 38.8% in the United States of America (USA) [151]. Moreover, as previously described, several strains of *S. aureus* are also resistant to methicillin (MRSA) and infections caused by MRSA are becoming a major concern worldwide [124, 125, 126, 152]. This increasing incidence of antibiotic resistant bacteria justifies the need to develop alternative antibiotics to treat impetigo and other SSSIs [128].

4.4 Topical antibiotics

4.4.1 Introduction

Topical application of antimicrobial agents may represent a useful tool for therapy ABSSSIs and has several potential advantages compared to systemic therapy [153]. Firstly, it can avoid unnecessary exposure of the gut flora to the antibiotic which may exert selection for resistance. Secondly, it is expected that the high local drug concentration in topical application and the negligible systemic absorption observed in most cases should overwhelm many mutational resistances. Thirdly, topical applications are less likely than

systemic therapy to cause side effects [154]. Topical antibacterial agents are commonly used in clinical practice for a variety of potential uses, which may include both prophylaxis and treatment of cutaneous bacterial infections (including impetigo), secondarily impetiginized eczematous dermatoses, staphylococcal nasal carriage, non-infectious dermatoses, and prophylaxis against of postoperative infections of surgical wounds and chronic wounds such as leg ulcers. The topical route of application offers several advantages over systemic administration, including the avoidance of systemic toxicity and side effects, the decreased induction of bacterial resistance, and the high concentration of antibacterial agent at the site of infection. Although, the potential risk of developing bacterial resistance has been suggested for the use of topical agents (especially with the older ones), also a lower risk for the development of bacterial resistance has been potentially associated with new agents [155].

However, a treatment that must be physically applied to the skin is limited by patient compliance, local side effects such as allergic contact dermatitis, and the depth of penetration of the agent its absorption and presentation with of potential systemic effects [156].

4.4.2 INDICATIONS OF USE

The specific antibiotics should be chosen for use according to the organism to be targeted, the location of the application, and other specific activities unique to each antibiotic class [157]. Some of the topical antibiotics most commonly used in dermatology together with their main indications are shown in the table below [157].

Table 10. Topical antibiotics and main dermatologic indications

Product	Indications*
Bacitracin ointment	Prevention of skin infections after minor compromise of skin integrity, such as burns, abrasions, or minor surgical procedures
Bacitracin zinc ointment	Prevention of skin infections after minor compromise of skin integrity, such as burns, abrasions, or minor surgical procedures
Triple antibiotic ointment (bacitracin zinc/neomycin/polymyxin B sulfate ointment)	Prevention of skin infections after minor compromise of skin integrity, such as burns, abrasions, or minor surgical procedures
Gentamycin 0.1% cream/ointment	Treatment of minor bacterial skin infections, including folliculitis, furunculosis, impetigo, eczema, infectious eczematoid dermatitis, and secondarily infected dermatoses
Mupirocin 2% cream	Topical treatment of secondarily infected traumatic skin lesions due to susceptible strains of *S. aureus* and *S. pyogenes*
Mupirocin 2% ointment	Topical treatment of impetigo due to: *S. aureus* and *S. pyogenes*

Product	Indications*
Retapamulin 1% ointment	Treatment of impetigo due to *S. aureus* (methicillin-susceptible isolates only) and *S. pyogenes* including: impetigo and infected small lacerations, abrasions, or sutured wounds
Topical clindamycin	Topical treatment of acne
Topical erythromycin	Topical treatment of acne
Topical sulfacetamide sodium	Topical treatment of acne and rosacea
Topical sulfacetamide sodium/sulfur	Topical treatment of acne and roscea
Topical tetracycline	Topical treatment of acne
Fusidic acid 2% cream	Treatment of primary and secondary skin infections caused by sensitive strains of *S. aureus*, *Streptococcus spp* and *C. minutissimum* including impetigo, folliculitis, sycosis barbae, paronychia and erythrasma, infected eczematoid dermatitis, infected contact dermatitis and infected cuts/abrasions
Fusidic acid 2% ointment	Treatment of primary and secondary skin infections caused by sensitive strains of *S. aureus*, *Streptococcus spp* and *C. minutissimum* including impetigo, folliculitis, sycosis barbae, paronychia and erythrasma, infected eczematoid dermatitis, infected contact dermatitis and infected cuts/abrasions
Nadifloxacin 1% cream	Topical treatment of inflammatory forms of acne *vulgaris* (papulospustular stage I-II)
*Indications may vary depending on the countries	

4.4.3 Safety and tolerability of topical antibiotics

The safety and tolerability profile of the most commonly used topical antibiotics for the treatment of impetigo and other SSSIs is generally good with no serious adverse reactions associated with their use in these indications. Most of the described adverse reactions are rare and related to the topical site application of the product.

The tables below describe the adverse reactions related to the most commonly prescribed topical antibiotics for the treatment of SSSIs.

Fusidic acid

Fusidic acid (-(acetyloxy)-5,17-dihydroxy-2,6,10,11-tetramethyltetracyclo[8.7.0.02,7.011,15]-heptadecan-14-ylidene]-6-methylhept-5-enoic acid) is an antibiotic derived from the fungus *Fusidium coccineum.*

The table below describes the adverse reactions most commonly associated with Fusidic acid [158, 159].

Table 11. Fusidic acid 2% cream, fusidic acid 2% ointment adverse reactions

SOC	Adverse reactions	Frequency*
Immune system disorders	Hypersensitivity	Rare
Eye disorders	Conjunctivitis	Rare
Skin and subcutaneous tissue disorders	Dermatitis (incl. dermatitis contact, eczema), rash, pruritus, erythema	Uncommon
	Angioedema, urticarial, blister	Rare
General disorders and administration site conditions	Application site pain (incl. skin burning sensation), application site irritation	Uncommon
Pediatric population	frequency, type and severity of adverse reactions in children are expected to be the same as in adults	
*The following convention has been used for the classification of frequency: very common: >1/10; common: >1/100 and <1/10; uncommon: >1/1,000 and <1/100; rare >1/10,000 and <1/1,000; very rare: <1/10,000		

Mupirocin

Mupirocin (9-[(E)-4-[(2S,3R,4R,5S)-3,4-dihydroxy-5-[[(2S,3S)-3-[(2S,3S)-3-hydroxybutan-2-yl]oxiran-2-yl]methyl] oxan-2-yl]-3-methylbut-2-enoyl]oxynonanoic acid) is a monoxycarbolic acid class antibiotic

The table below describes the adverse reactions most commonly associated with Mupirocin [160, 161, 162, 163].

Table 12. Mupirocin 2% cream, mupirocin 2% ointment adverse reactions

SOC	Adverse reactions	Frequency*
Immune system disorders	Systemic allergic reactions such as generalized rash, urticaria and angioedema	Very rare
Skin and subcutaneous tissue disorders	Application site hypersensitivity reactions including urticaria, pruritus, erythema, burning sensation, contact dermatitis, rash	Common
	Itching, erythema, stinging and dryness localized to the area of application have been described for the ointment formulation	Uncommon
	Cutaneous sensitization reactions to mupirocin or the ointment base	Uncommon
Pediatric population	Skin dryness and erythema	Unknown
*The following convention has been used for the classification of frequency: very common: >1/10; common: >1/100 and <1/10; uncommon: >1/1,000 and <1/100; rare >1/10,000 and <1/1,000; very rare: <1/10,000		

Retapamulin

Retapamulin ((3aS,4R,5S,6S,8R,9R,9aR,10R)-6-ethenyl-5-hydroxy-4,6,9,10-tetramethyl-1-oxodecahydro- 3a,9-propano-3aH-cyclopenta[8]annulen-8-yl {[(1R,3s,5S)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl]} sulfanyl}acetate) is a pleuromutilin antibiotic.

The table below describes the adverse reactions most commonly associated with Retapamulin [164, 165].

Table 13. Retapamulin 1% ointment adverse reactions

SOC	Adverse reactions	Frequency*
Immune system disorders	Hypersensitivity, angioedema	Unknown
Skin and subcutaneous tissue disorders	Contact dermatitis	Uncommon
General disorders and administration site conditions	Irritation	Common
	Application site pain, pruritus, erythema	Uncommon
Pediatric population	frequency, type and severity of adverse reactions in children are expected to be the same as in adults	
*The following convention has been used for the classification of frequency: very common: >1/10; common: >1/100 and <1/10; uncommon: >1/1,000 and <1/100; rare >1/10,000 and <1/1,000; very rare: <1/10,000		

Nadifloxacin

Nadifloxacin ((RS)-9-Fluoro-8-(4-hydroxy-piperidin-1-yl)-5-methyl-1-oxo-6,7-dihydro-1H,5H-pyrido[3,2,1-ij]quinoline-2-carboxylic acid) is a fluorinated quinolone available in some markets in the form of a 1% cream for the topical treatment of mild and moderate inflammatory forms of acne vulgaris (papulospustular stage I-II).

The table below describes the adverse reactions most commonly associated with Nadifloxacin [166].

Table 14. Nadifloxacin 1% cream adverse reactions

SOC	Adverse reactions	Frequency*
Skin and subcutaneous tissue disorders	Pruritus	Common
	Papules, dryness of the skin, contact dermatitis, itching, skin warmth, erythema	Uncommon
	Urticaria, skin hypopigmentation	Very rare
*The following convention has been used for the classification of frequency: very common: >1/10; common: >1/100 and <1/10; uncommon: >1/1,000 and <1/100; rare >1/10,000 and <1/1,000; very rare: <1/10,000		

4.5 THE SKIN BARRIER

The skin is an organ of the integumentary system made up of multiple layers of ectodermal tissue which act as an anatomical barrier from pathogens and damage between the internal and external environment [167].

It is the largest organ of the body, accounting for about 15% of the total body weight in adult humans. It exerts multiple vital protective functions against environmental aggressions, rendered possible thanks to an elaborate structure, associating various tissues of ectodermal and mesodermal origin, arranged in three layers, including (from top to bottom) the epidermis (and its appendages), the dermis and the hypodermis [168].

Normal, healthy skin is a natural physical barrier to bacterial invasion. An intact stratum corneum layer provides a barrier to a wide variety of bacteria and other pathogens. The natural resistance of the skin to bacterial penetration and multiplication is not completely understood, but some elements involved include the following factors [158]:

- Inability of organisms to penetrate the keratinized stratum corneum.
- Desquamation, which sheds bacteria as it sloughs keratinocytes.
- Natural acidity of the skin (pH 5.5).
- Presence of antibacterial substances in sebaceous secretions and intracellular lipids of the stratum corneum.
- Relatively low moisture content of skin.
- Normal cutaneous microflora.

Changes in any of these factors together with changes in the overall ability of the host to mount an inflammatory response can have an influence on individual's susceptibility to infection, Nonpathogenic infectious agents can become disease-producing pathogens in individuals with reduced cellular or humoral defenses or defects (e.g. immunocompromised or nutritionally compromised subjects).

The epidermal layer of the skin has not only a defensive and protective function but also its barrier function avoids water loss and favors the colonization by normal flora while avoiding the development of pathogens [169, 170]. The stratum corneum, the external layer of the epidermis is a layer of dead cells lacking nucleus.

Desquamation, the process of cell shedding from the surface of the stratum corneum, balances proliferating keratinocytes that form in the basal stratum. During cornification, living keratinocytes are transformed into dead corneocytes; the cell membrane is replaced by a layer of ceramides which become covalently linked to an envelope of structural proteins (the cornified envelope). Desquamation and formation of the cornified envelope are both required for the maintenance of skin homeostasis. The cells of the stratum

corneum contain a dense network of keratin, a protein that helps keep the skin hydrated by preventing water evaporation [171, 172].

There are significant structural and functional differences between adult and infant skin which suggest a greater susceptibility of infants to both percutaneous absorption of product and penetration of UV light [173]. The stratus corneum although, present at birth, gains thickness, hydration capacity, and acidification throughout infancy [173, 174, 175]. Infants and toddlers (to 33 months) show higher water content throughout their strum corneum than adults despite a lower concentration of natural moisturizing factors. Skin capacitance, trans-epidermal water loss, and water absorption-desorption rates (both absorption and loss of water) showed consistently higher values throughout the first year of life in comparison with older children (up to 4 years of age), particularly with adults [176]. Therefore the skin surface morphology, desquamation, and epidermal expression of keratins and other proteins reflect a compromised barrier integrity in young children compared to older children and adults [177, 178, 179]. The stratum corneum and total epidermis are thinner in infants than in adults due to a smaller and denser layer of corneocytes and keratinocytes suggesting a faster turnover rate and greater surface area contribute to increased water absorption. Some studies have suggested a relatively lower melanin concentration in infants and toddlers versus adults [180].

Apart of the considerations previously mentioned (organism to be targeted, location of the application, specific activities unique to each antibiotic class), the selection of an antibiotic agent to be applied topically as well as the kind of topical formulation, should also take into account the mentioned characteristics of the skin.

4.6 DEVELOPMENT OF TOPICAL PRODUCTS

The development of a topical product for dermal administration should consider many different factors [173]:

- What is the optimum formulation?
- Are the Active Pharmaceutical Ingredient (API) and excipients stable in the formulation?
- What is the optimum concentration of the API?
- Does the API need to be absorbed to be active systemically, or is its activity solely a local effect?
- If the effect is intended locally, what level in the skin does this effect take place?
- Is the API absorbed and what happens after absorption in terms of pharmacokinetics and in situ toxicokinetics?

- Are the API and excipients likely to be locally toxic or irritant or cause sensitization and allergic reactions? As it is applied to skin which may well be exposed to sunlight, is there any potential for phototoxicity or photosensitization.
- Have packaging and container closures been given adequate consideration?
- What effect does the API have, what are we treating, what are we able to measure in terms of efficacy and safety?
- Do the measurements used provide reliable, accurate, and reproducible results? Any such measurements need to take into account the condition being treated and also any other underlying pathology which could be relevant or have an effect on the pharmacokinetics or pharmacodynamics.

When considering the choice of a topical formulation for dermal administration, it has to be taken into account that changes in the concentration of the API, or the excipients selected can influence the stability, or the pharmacokinetics of the preparation and it is therefore important to take that into account in an early stage [181]. The choice of formulation is normally based in factors such as stability and compatibility of the API in the vehicle as well as potential patient acceptability. However, some recent studies have shown that the choice of excipient(s) clearly influences the fate of the active in skin [181, 182, 183, 184] and that also needs to be taken into account. Different vehicles can be used for topical delivery of drugs such as powders, creams, ointments, gels, sprays, foams and patches. However, in the selection of the formulation, patient preference will still be an important factor to take into account [181]. The selection of excipients should also be made carefully. For instance, some formulations like creams are oil and water mixtures and require emulsifiers, stabilizers and preservatives to make them stable but some of these compounds are often irritant to skin and potential sensitizers, which can be a problem for the objective in the treatment of skin diseases [185]. An example is DMSO (Dimethyl sulfoxide) which is used as a penetration enhancer for some active drugs and may produce itching, burning, erythema and urticarial [186].

Dermatology indications face some particular challenges when it comes to drug development. The assessment of bioavailability of topical drugs and its utility in weighing benefits versus risks is unique and challenging as compared with assessments for conventional systemic drugs. For a topical drug development, drug exposure–response relationship and drug disposition requires a quite different perspective than that for systemic oral drugs [187]. Bioavailability, defined in the FDA Code of Federal Regulations 21 CFR 320.1(a) as "the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action" [188] is a much more complex issue when evaluating topical agents as plasma levels of topically applied, locally acting products are not representative of true bioavailability because they do not represent the concentrations at the site of action. At the contrary, systemic absorption in

this case may represent in many cases potential unwanted pharmacological and/or toxicological effects in unintended organ tissues and thus may raise safety concerns. For topical drugs, measurement of pharmacokinetic properties directly on or in skin would be more meaningful because it would address drug disposition properties in the compartments that are proximal to the target and therapeutically relevant. Regulatory Authorities (FDA, EMA) often require the evaluation under "maximal-usage" conditions to evaluate systemic availability of topically applied, locally acting drug products (e.g. highest strength of the proposed product/formulation at the highest proposed posology in extreme conditions such as abraded skin and in patients with the target indication). Since, changes in product formulations and/or excipients could greatly affect the extent of absorption for the same active ingredient, it is critical and required by Regulators to use the final to-be-marketed product in bioavailability studies conducted in patients with the target indication and in the pivotal phase III studies [187].

The development of a new drug requires the standard preclinical pharmaceutical and toxicological development before administration to humans in Phases I, II and III clinical trials. In the case of topical drugs some of the preclinical toxicological studies may be conducted by a systemic route to evaluate the toxicological effects under a high exposure and also by the topical route of administration (e.g. dermal) to evaluate the local as well as systemic effects. During the clinical development of a topical product for dermal application, besides the standard phase I, II, and III clinical trials to evaluate the absorption and pharmacokinetics, safety, and tolerability, effective dose, and efficacy, specific clinical studies should be conducted to test the dermal tolerability profile of the API and excipients (potential to induce irritation, sensitization, phototoxicity, and photoallergy). Dermal pharmacokinetic studies may also be needed to understand the metabolism of the drug in the skin [181]. Depending on the indication, a pediatric development plan should be conducted which may include the development of pediatric formulation specific for different pediatric age groups.

The regulatory requirements in Europe are covered by Directive Regulatory 2001/83/EC and successive amendments [189]. The Pediatric Regulation is comprised of: the Regulation (EC) No 1901/2006 [190] and Regulation (EC) No 1902/2006 [191]. In USA, the primary statutory basis for drug approval is the 1938 Federal Food, Drug, and Cosmetic Act which have been amended by the Kefauver-Harris amendments which mandate that the FDA must determine that a drug product is both safe and effective before it may be approved for marketing [192, 193]. The Pediatric Regulation is comprised in the Pediatric Research Equity Act [194] and in the Best Pharmaceuticals for Children Act [195]. Specific recommendations and guidance documents for the different phases of development and for specific indications are also provided by the EMA (www.ema.europa.eu) and the FDA (www.fda.gov).

4.7 DRUGS IN DEVELOPMENT FOR DERMATOLOGICAL INFECTIONS

A variety of products is currently in development for the treatment of ABSSSIs. These include new chemical entities (obtained by chemical synthesis or protein derivate products) and new formulation of old products (including fixed dose combinations) with different mechanisms of action and routes of administration. Many of the products are potentially targeting MRSA infections [196, 197, 198].

A summary of drugs in development for dermatological infections is included in table below including status of development, originator company, licensee company, main dermatological infection indication, therapeutic class, mechanism of action and route of administration.

Table 15. Drugs in development for ABSSSIs

Drug Name	Status	Originator	Licensee	Indication*	Therapeutic Class	Mechanism Action	Route
Zabofloxacin	Phase III	Dong Wha	IASO Pharma Pacific Beach Bio Sci.	ABSSI	Fluoro-quinolone	DNA gyrase and topoisomerase inhibitor	Oral
Fusidic acid, CEM-102	Phase III	Cempra		ABSSSI, MRSA	Bacteriostatic antibiotic	Protein synthesis inhibitor	Oral
Delafloxacin	Phase III	Wakunaga	Melinta, Abbott Eurofarma	ABSSSI, MRSA	Fluoro-quinolone	DNA gyrase and topoisomerase inhibitor	Oral
Auriclosene, NVC-422	Phase III	NovaBay	Galderma, Novartis	Impetigo, MRSA	Aganocide antimicrobial	Analogue of the amino-acid derivative N-chlorotaurine	Topical
TD-1792	Phase II	Theravance	R-Pharm	ABSSSI, MRSA	Heterodimer antibiotic	Cell wall synthesis inhibitor	IV
Radezolid	Phase II	Melinta Therapeutics		ABSSSI, MRSA	Oxazolidinone	Protein 30S and 50S ribosomal subunit inhibitor	Oral
Omadacycline amadacycline, BAY 73-6944	Phase III	Paratek	Novartis, Merck, Bayer, GSK	ABSSSI, MRSA	Tetracycline	Protein 30S ribosomal subunit inhibitor	Oral
MRX-I	Phase II	MicuRx		ABSSSI, MRSA	Oxazolidinone	Protein 50S ribosomal subunit inhibitor	Oral
Minocycline, FDX-104	Phase II	Foamix		Rosacea, impetigo, acne, ABSSI	Tetracycline	Protein 30S ribosomal subunit inhibitor	Topical
Lysostaphin	Phase II	Bharat Biotech		ABSSSI	S. simulans metallo-endopeptidase	Cell wall synthesis inhibitor	Topical
LTX-109, Lytixar	Phase II	Lytix Biopharma		Impetigo, ABSSSI, MRSA	Membrane-degrading peptide	Membrane integrity inhibitor, membrane permeability enhancer	Topical

Drug Name	Status	Originator	Licensee	Indication*	Therapeutic Class	Mechanism Action	Route
Lefamulin, BC-3781	Phase II	Nabriva Therapeutics	Forest	ABSSSI, MRSA	Pleuromutilin	Protein 50S ribosomal subunit inhibitor	Oral
GSK-2140944	Phase II	GSK		ABSSSI	Topoisomerase-II Inhibitor	DNA topoisomerase II inhibitor	Oral
DPK-060	Phase II	Pergamum		ABSSI, MRSA	Human kininogen derived peptic antibiotic	Unidentified pharmacological activity	Topical
Debio-1452, AFN-1252	Phase II	Affinium	Debiopharm	ABSSI, MRSA	Antibacterial	Enoyl-acyl carrier protein reductase (FabI) inhibitor	Oral
CG-400549	Phase II	Crystal-Genomics		ABSSI, MRSA	Antibacterial	Enoyl-acyl carrier protein reductase (FabI) inhibitor	Oral
Ceftriaxone + tazobactam	Phase II	China-Pharma Holdings		ABSSSI	Fixed dose combination B-lactamase inhibitors	Cell wall synthesis inhibitor, Lactamase-B inhibitor	Oral
Brilacidin, PMX-10066	Phase II	PolyMedix	Dr Reddy's	ABSSI, MRSA	Polymer-based antibiotic	Defensin agonist Membrane integrity inhibitor	IV
Avibactam + ceftaroline acetate, CEF104	Phase II	Forest Laboratories	Astra-Zeneca	ABSSI, MRSA	Fixed dose combination B-lactamase inhibitors	Lactamase-A and -C inhibitor	IV
Acorafloxacin, JNJ-32729463	Phase II	Johnson & Johnson	Furiex	ABSSI, MRSA	Fluoro-quinolone	DNA gyrase and topoisomerase inhibitor	Oral
BC-7013	Phase I	Nabriva Therapeutics		ABSSSI, MRSA, acne	Pleuromutilin	Protein synthesis inhibitor	Topical
RX-02	Preclinical	Melinta Therapeutics		ABSSI, MRSA	Macrolide	Protein 50S ribosomal subunit inhibitor	Oral
KBP-7072	Pre-clinical	KBP Biosciences		ABSSSI	Tetracycline	Protein ribosomal inhibitor	NA
IOG-101, DBAF-101	Pre-clinical	ioGenetics		ABSSI, MRSA	Fusion protein antibacterial	Unidentified pharmacological activity	NA
Ozenoxacin	Phase III	Ferrer		Impetigo	Non-fluorinated quinolone	DNA gyrase and topoisomerase inhibitor	Topical
* Dermatological infection indications in development (other indications may also be in development for some compounds)							

4.8 OZENOXACIN

4.8.1 INTRODUCTION

Ozenoxacin (GF-001001-00) (1-cyclopropyl-8-methyl-7-(5-methyl-6-methylamino-pyridin-3-yl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid) is an antibacterial agent that belongs to the chemically related family of quinolones and has potent bactericidal effect against pathologically relevant Gram-positive strains. ozenoxacin is currently being developed by Ferrer as a topical formulation (cream), containing 1% of active ingredient (ozenoxacin) for the topical treatment of uncomplicated skin and skin structures bacterial infections. The main indication for which the product is developed is impetigo in adults and children (aged 2 months and older).

Ozenoxacin is a non-floorinated quinolone and the lack of a halogen substituent at the 6-position clearly differentiates ozenoxacin from most of the antibacterial quinolones, which are fluoroquinolones and present some safety concerns. ozenoxacin shows a dual target of action, inhibiting DNA gyrase and topoisomerase IV [199]. ozenoxacin shows activity against *S. aureus* strains *(S. aureus SA113 mutants)* that carry mutations of the QRDRs encoding the A subunits of the DNA gyrase and topoisomerase IV and resistant to fluoroquinolones such as ciprofloxacin and moxifloxacin [199].

In order to evaluate the use of topical ozenoxacin for the treatment of acute bacterial skin and skin structure infections (ABSSSIs), a thorough preclinical characterization, in terms of active pharmaceutical ingredient and formulation properties, pharmacology, pharmacokinetics and toxicology has been conducted. All these studies have demonstrated a potent bactericidal effect against pathologically relevant Gram-positive bacteria responsible for ABSSSIs, especially *Staphylococci* and *Streptococci* strains (including strains resistant to other antibiotics), a low potential of absorption after topical applications, and lack of toxicological effects that are usually associated with fluorinated quinolones (e.g. photosensitivity and phototoxicity reactions, and chondrotoxicity).

As part of the clinical development and to ensure the safety and efficacy in humans, topical ozenoxacin has been evaluated in controlled clinical trials in healthy volunteers as well as in secondarily infected traumatic lesions (SITLs) and in adult and pediatric patients with impetigo. Bioavailability, general safety and tolerability and specific local (dermal) tolerability studies in phase I clinical trials conducted in healthy adults have been conducted with different topical formulations, concentrations, and administration regimens applied to different skin extensions, and to normal and damaged skin showing no absorption and absence of any relevant safety and tolerance issues. Absorption, safety and tolerability has also been evaluated in a phase I-II clinical trial in adult and pediatric patients (aged 2 months and older) with impetigo has shown similar results than studies in

healthy volunteers and has provided evidence of the clinical effect of ozenoxacin in this patient population.

The clinical and microbiological efficacy of ozenoxacin in the treatment of infections caused by *S. aureus* and S. *pyogenes* have been demonstrated in a phase II clinical trial conducted in adult patients with secondarily infected traumatic lesions (SITLs), and two phase III clinical trials conducted in adult and pediatric patients (the first one included patients aged 2 years and older and the second one included patients aged 2 months and older). The phase II and the two phase III clinical trials have also demonstrated a very good safety and tolerability profile in adult and pediatric patients (aged 2 months and older). All these studies complete the data for the dossier needed for regulatory approval of the product.

The characterization of ozenoxacin in terms of spectrum of antibacterial activity has shown potent bactericidal effect against pathologically relevant Gram-positive strains, especially Staphylococci and Streptococci ones, and including strains resistant to other antibiotics. Escalating numbers of these bacteria, especially Staphylococcus aureus, have become resistant to leading topical antimicrobials used in human clinical practice. For instance *S. aureus* has shown resistance to agents such as mupirocin, ranging from 2% to 28% [200], and fusidic acid, ranging from 6% to 50% [146].

Ozenoxacin is currently being developed as a topical formulation (cream), containing 1% ozenoxacin for the topical treatment of uncomplicated skin and skin structures bacterial infections including impetigo. The main indication for which the product is developed is impetigo in both adults and children (aged 2 months and older). The inactive excipients included in the formulation are known, commonly used in topical formulations, and have been selected according to results of a drug excipient compatibility study.

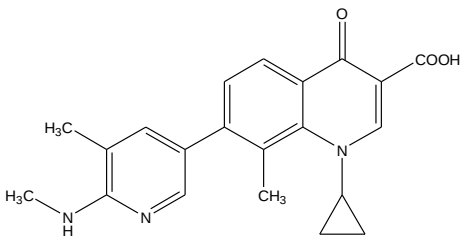
In order to evaluate the use of topical ozenoxacin for the treatment of ABSSSIs, a thorough preclinical characterization, in terms of active pharmaceutical ingredient and formulation properties, pharmacology, pharmacokinetics and toxicology has been conducted. To ensure the safety and efficacy in humans, topical ozenoxacin has been evaluated in controlled clinical trials in healthy volunteers as well as in SITLs and patients with impetigo. Bioavailability, safety and local tolerability studies on healthy adults and adults and pediatric patients have been already completed with different topical formulations, skin extensions and dosages without any relevant safety and tolerance issues.

4.8.2 ACTIVE SUBSTANCE

Chemical name (IUPAC): 1-Cyclopropyl-8-methyl-7-(5-methyl-6-methylamino-pyridin-3-yl)-4-oxo-1,4-dihydro-quinoline-3carboxylic acid

INN name: ozenoxacin.

Structure:



Molecular formula: C21H21N3O3.

Formulation in development: 1% cream.

4.8.3 PRECLINICAL DATA

4.8.3.1 MECHANISM OF ACTION

Ozenoxacin has demonstrated to be potent selective inhibitor of DNA replication, blocking the bacterial DNA gyrase and the topoisomerase IV enzymes. When compared to other quinolones (nadifloxacin, ofloxacin, levofloxacin), ozenoxacin shoes a greater inhibitory activity for both enzymes, reflected by lower minimum inhibitory concentration (MIC) and half inhibitory concentration values (IC_{50}) values. ozenoxacin retains activity against *S. aureus* strains that carry mutations in QRDR encoding the A subunits of the DNA gyrase and topoisomerase IV, the protein targets for quinolones and thereby resistant to fluoroquinolones such as ciprofloxacin and moxifloxacin [199, 201].

4.8.3.2 SPECTRUM OF ANTIBACTERIAL ACTIVITY

Ozenoxacin has demonstrated a potent antibacterial activity in different *in-vitro* conducted against different pathogens including strains from different geographical areas and comparison with other antibiotics. The potent activity has also been demonstrated in preclinical studies conducted in *in-vivo* experimental models of infection.

a) *In-vitro* studies

Ozenoxacin has shown activity against Gram-positive organisms, resistant strains to other antibiotics commonly used in ABSSSIs (e.g. mupirocin and fusidic acid) and against, methicillin-, penicillin- and/or ciprofloxacin-resistant strains. ozenoxacin has showed the greatest antibacterial activity when compared to these other antibiotics [202].

Strong activity active has been demonstrated against strains of *Group B Streptococci, Group C Streptococci, B. fragilis and C. perfringens. Aside from these isolates, ozenoxacin has improved activity compared to all fluoroquinolones comparators amongst all strains of MRSA, MSSA, MRSE, MSSE, Groups A and G Streptococci, S. constellatus, S. bovis, S. mitis, S. oralis, S. pneumoniae and Clostridium difficile*. A higeher spectrum of activity has

been shown in comparison to other compounds (e.g. ciprofloxacin, levofloxacin, moxifloxacin, amoxicillin, amoxicillin plus clavulanic acid, ceftriaxone, tigecycline, linezolid, daptomycin and clarithromycin) [203].

A potent activity has also been demonstrated against *MRSA, MSSA, S. epidermidis, S. pyogenes, S. pneumoniae and other beta-haemolytic Streptococci, Corynebacterium spp, Micrococcus spp and Lactobacillus spp* when compared to amoxicillin-clavulanate, ceftriaxone, daptomycin, clindamycin, erythromycin, gentamicin, levofloxacin, vancomycin, linezolid, ciprofloxacin, mupirocin, retapamulin, fusidic acid, neomycin, tetracycline, cefurosime, penicillin [204].

In a study conducted with 1054 isolates from 35 countries worldwide, ozenoxacin has demonstrated strong activity against quinolone–susceptible *Staphylococci*. It was also very active against *S. pneumoniae and beta-hemolytic streptococci*, quinolone-susceptible *Enterococci* (*E. faecalis*). Altogether, in Gram-positive bacteria, ozenoxacin MIC was higher against those isolates non-susceptible to quinolone. It was also very active against other Gram-negative species as *M. catarrhalis or H. influenza* [205].

The antimicrobial activity of ozenoxacin against *S.aureus* strains has not been affected by the presence of virulence factors associated with higher pathogenicity such as Phenol Soluble Modulin (PSM) genes and/or production Panton-Valentine leukocidin (PVL) virulence factors [206].

Ozenoxacin has demonstrated to have a bactericidal activity against different isolates of *S. aureus, S. epidermidis, S. pyogenes and S. pneumonia*. Methicillin resistant *Staphylococci*, penicillin resistant *Pneumococci* and erythromycin resistant *S. pyogenes* did not affect the bactericidal activity of ozenoxacin. The activity was concentration-dependent more active than levofloxacin (when measured on a weight by weight basis) [207].

Ozenoxacin has also shown excellent *in-vitro* activity against the most important microorganisms isolated as etiological agents in skin infections, even against methicillin-resistant strains showing 2, 3 or 4 mutations in the gyrA and/or parC genes. The rate of selection of resistant mutants of ozenoxacin is lower than that observed with levofloxacin and ciprofloxacin in Methicillin-Susceptible *Staphylococcus aureus* (MSSA) and Methicillin-Susceptible *Staphylococcus epidermidis* (MSSE) isolates with or without a mutation in the gyrA gene. However, this rate is similar to that of Levofloxacin and lower than that found with ciprofloxacin in MSSA, MRSA and MSSE isolates with more than one mutation in the gyrA and/or parC genes and also in strains of *S. pyogenes, S.agalactiae* and *E. faecium*, independently of the mutations presented. The mutant prevention concentration of ozenoxacin was lower compared to that of Levofloxacin and ciprofloxacin in MRSA, MSSA, MRSE and MSSE strains with one or more mutations in the gyrA and parC genes. A significant higher inhibitory activity of ozenoxacin for the DNA gyrase of *S.aureus* compared to levofloxacin, moxifloxacin and ciprofloxacin has been observed.

This is in line with the better in vitro activity showed by ozenoxacin compared to the other quinolones. ozenoxacin shows a similar inhibitory activity against topoisomerase IV than moxifloxacin and ciprofloxacin and better than levofloxacin [208].

b) *In-vivo* studies

Ozenoxacin has also shown potent activity when studied with different topical formulations (1% gel, 1% ointment, 1% ointment) and administration regimens using a dermal infection model by *S. aureus* in mice. The antibacterial activity was higher that other antibacterials tested in the model (e.g. mupirocin 2% ointment, retapamulin 1% oitment) [209, 210].

4.8.3.3 SAFETY PHARMACOLOGY

In-vitro and *in-vivo* safety pharmacology studies for ozenoxacin have been completed to assess the effect on cardiovascular system (including evaluation of the effect on the QT interval), respiratory function and CNS activity.

a) *In-vitro* studies

To evaluate the potential cardiac effects (potential blockage of K channels), different concentrations of ozenoxacin were evaluated in and *in-vitro* model of cloned human-ether-à-go-go-related gene (hERG) potassium channels expressed in mammalian cells (hERG in voltage clamped human embryonic kidney cells (HEK293)). The higher concentrations of ozenoxacin showed inhibitory effects on hERG which were significantly higher than vehicle control values [211]. Inhibitory effects on hERG indicate a blockage of K channels that can potentially be translated clinically on a prolongation of the QT interval on the electrocardiogram and, on rare occasions, ventricular arrhythmia. Although not considered specifically a class effect, blockade of the human cardiac K channel HERG has been described with other quinolones [212].

b) *In-vivo* studies

The details of the *in-vivo* safety pharmacology studies are included in the table below.

Table 16. *In-vivo* safety pharmacology studies

Test	Species	Route	Ref.
Cardiovascular system	Dog	Oral	[213]
QT-interval	Guinea pig	IV	[214]
Respiratory system	Rat	Oral	[215]
Irwin test	Rat	Oral	[216]

The effect on the cardiovascular system and ECG different oral concentrations of ozenoxacin were evaluated in beagle dogs. No systematic inductions of either conduction disturbances or QT prolongation were found even at very high doses [213].

The potential cardiac effects on ECG QT-interval were also evaluated by IV infusion of rising doses of ozenoxacin to anaesthetized Guinea pigs. There was a tendency to a shorten P and PR interval after administration and slight tendency to lengthen QRS interval during endovenous infusion and no prolongation of QT-interval (even at high doses) [214].

No effect on respiratory parameters were shown after administration of increasing doses of ozenoxacin to conscious rats [215].

An Irwin test to evaluate the effects on general physiological function and behavior conducted with increasing oral doses of ozenoxacin in rats showed no effect on behavioral or physiological parameters [216].

4.8.3.4 Toxicology

The toxicology assessment of ozenoxacin was initially designed to support phase I clinical trials with and included a single dose IV study in the rat, a dose-range finding oral study in dogs, and two 28-days dermal repeated dose studies in the rat and minipigs. These studies allowed the initiation of the clinical development program and the administration of ozenoxacin to healthy volunteers in phase I clinical trials.

Additional toxicology studies were conducted to support the clinical development of ozenoxacin. These studies were conducted with different formulations, dose administrations (single and repeated administrations) including very high doses, different routes of administrations, and with different animal species. These studies were conducted to characterize the toxicological effects of ozenoxacin. Although, the intended route of administration is topical, some of the toxicological studies were conducted with oral or IV administration in order to guarantee an important systemic exposure.

As requested by Regulatory Authorities a 28-days dermal toxicology study in minipigs on intact and abraded s was conducted to evaluate the absorption, safety and tolerability of the ozenoxacin cream formulation that was being evaluated in clinical trials.

Reproductive toxicology studies were conducted including included developmental toxicity, fertility, perinatal/postnatal reproductive, toxicity, and genotoxicity studies.

Furthermore, as an increased risk of developing tendon and articular-cartilage has been associated with some quinolones (ciprofloxacin, levofloxacin and moxifloxacin), toxicity studies in juvenile animals were conducted to evaluate the potential skeletal and/or articular effects of ozenoxacin.

The details of the principal toxicological studies are summarized below.

a) Single-dose studies

The details of the single-dose toxicity study are included in the table below.

Table 17. Single-dose toxicity study

Species	Route	Duration	Ref.
Rat	IV infusion	Single dose	[217]

As single raising-dose study was conducted in rats with IV administrations of ozenoxacin. High dose administrations were related to clinical signs such as dyspnea, salivation, pigmented lacrimation, vocalization, tremors, convulsions, twitches, excitation and prostration [217]. These effects are normally observed at high doses in this kind of study.

b) Dose-ranging studies

The details of the single-dose toxicity study are included in the table below.

Table 18. Dose-ranging toxicity study

Species	Route	Duration	Ref.
Dog	Oral	≤ 14 days	[218]

A dose-ranging study was conducted in beagle dogs with oral administrations of ozenoxacin. High doses induced gastrointestinal disorders, convulsions and mortality (as expected in this kind of study). The study allowed choosing the doses for 4-week repeated administration toxicity studies [218].

c) Repeated-dose studies

The details of the repeated-dose toxicity studies are included in the table below.

Table 19. Repeated-dose toxicity studies

Species	Route	Duration	Ref.
Rat	Dermal	28 days	[219]
Minipig	Dermal	28 days	[220]
Minipig	Dermal (intact & abraded skin)	28 days	[221]
Rat	Oral	28 days	[222]
Dog	Oral	28 days	[223]

Repeated-dose administration studies (4-week administrations) were conducted in different species (rat, minipig, dogs) and different routes of administration (dermal, oral).

A repeated-dose with increasing doses of ozenoxacin ointment applied topically during 28 days was conducted in rats. ozenoxacin was well tolerated locally and systemically. The high doses affected gastrointestinal system, body weight, globulin, and organ weight [219]. These effects are normally observed at high doses in this kind of study.

A repeated-dose with increasing doses of ozenoxacin ointment applied topically during 28 days was conducted in minipigs. ozenoxacin was well tolerated locally and systemically [220].

A repeated-dose with increasing doses of ozenoxacin ointment applied topically to intact and abraded skin during 28 day was conducted in minipigs. ozenoxacin was well tolerated locally and systemically with no adverse effects toxicologically relevant. The toxicokinetic analyses showed no systemic exposure (no absorption of ozenoxacin through intact and abraded skin) [221].

A repeated-dose with increasing doses of oral ozenoxacin administrations during 28 days was conducted in rats. ozenoxacin was well tolerated systemically. No toxicologically relevant adverse effects. No significant histopathological findings were noted in the tissues evaluated. Toxicokinetic analysis showed satisfactory systemic exposure [222].

A repeated-dose with increasing doses of oral ozenoxacin administrations during 28 days was conducted in dogs. High doses were associated with cases of CNS effects and mortality. These effects are normally observed at high doses in this kind of study. Besides these effects no relevant effects on ophthalmology, electrocardiography, and clinical pathology parameters were recorded at any dose levels. Furthermore, no significant histopathological findings were noted in the tissues evaluated. Toxicokinetic analysis showed satisfactory systemic exposure [223].

d) Reproductive toxicity studies

Reproductive toxicity studies are conducted to evaluate the toxic effects of a substance on the sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring.

As required by guidelines for the development of medicinal products, reproductive toxicity studies were conducted with different oral doses of ozenoxacin (repeated administrations) in different species (rat, rabbit).

The details of the reproductive toxicity studies are included in the table below.

Table 20. Reproductive toxicity studies

Species	Route	Duration	Ref.
Rat	Oral	Gestation Days 7 to 17	[224]
Rat	Oral	Gestation Days 7 to 17	[225]
Rabbit	Oral	Gestation Days 7 to 19	[226]
Rabbit	Oral	Gestation Days 7 to 19	[227]
Rat	Oral	Males: 4-weeks prior mating until sacrifice; females: 2-weeks prior mating until gestation day 7	[228]
Rat	Oral	Gestation day 7 to day 20 postpartum	[229]

The reproductive toxicology studies demonstrated that after high oral dosages with adequate demonstrated systemic exposure, ozenoxacin did not induce adverse effects on male or female fertility, gestation, parturition, lactation or maternal behavior on F0 generation and did not affect reproductive parameters and growth pattern on F1 generation.

e) Genotoxicity studies

Genotoxicity tests are in vitro and in vivo tests designed to detect compounds that induce genetic damage by various mechanisms. These tests enable hazard identification with respect to damage to DNA and its fixation. Fixation of damage to DNA in the form of gene mutations, larger scale chromosomal damage, or recombination is generally considered to be essential for heritable effects and in the multistep process of malignancy, a complex process in which genetic changes might possibly play only a part. Numerical chromosome changes have also been associated with tumor-genesis and can indicate a potential for aneuploidy in germ cells. Compounds that are positive in tests that detect such kinds of damage have the potential to be human carcinogens and/or mutagens [230].

As part of the preclinical development of ozenoxacin different in-vitro and in-vivo studies were conducted to evaluate the genotoxic potential of ozenoxacin.

i) *In-vitro*-studies

Various o genotoxicity studies have been conducted in different *in-vitro* models.

The details of the *in-vitro* genotoxicity studies are included in the table below.

Table 21. *In-vitro* genotoxicity studies

Assay	Strain/cell	Ref.
Ames test	*Salmonella typhimurium, E. coli*	[231]
Mouse lymphoma assay	Mouse lymphoma L5178Y cells	[232]
Photogenotoxicity	Chinese Hamster Lung cells	[233]

The *in-vitro* genotoxicity studies conducted with different concentrations of ozenoxacin have shown no mutagenic and no photogenotoxic effects.

ii) *In-vivo* studies

The details of the *in-vivo* genotoxicity studies are included in the table below.

Table 22. *In-vivo genotoxicity* studies

Species	Route	Duration	Ref.
Rat	Oral	Two (separated by a 24 h interval)	[234]

An *in-vivo* genotoxicity conducted with different dose of oral administrations of ozenoxacin to rats has shown no cytotoxic effects to bone marrow. The toxicokinetic analyses showed no systemic exposure [234].

f) Toxicity studies in juvenile animals

The use of quinolones has been associated with an increased risk of developing tendon and articular-cartilage damage remain. This class adverse effect is reported in juvenile animals since the 1970's, with the first quinolone agent (nalidixic acid). In order to evaluate the potential effects on tendons and articular cartilages as well as in other organs several studies were conducted with ozenoxacin in juvenile animals.

The details of the toxicity studies conducted in juvenile animals are included in the table below.

Table 23. Toxicity studies in juvenile animals

Species	Route	Duration	Ref.
Juvenile rat	Oral	5-days	[235]
Juvenile dog	Oral	2-weeks	[236]

A repeated-dose toxicity study conducted in juvenile rats with oral administrations of ozenoxacin administered during 5 days in comparison to Ofloxacin (as active comparator) did not produce any observable articular lesion while the active control produced as expected articular lesions [235].

A repeated-dose toxicity study conducted in juvenile dogs with oral administrations of ozenoxacin y administered during 2 weeks did not show any relevant toxicologically effects. There were no relevant macroscopic or microscopic changes in the organs examined (brain, thymus, liver, lung and kidney). There was no microscopic evidence of quinolone-induced arthropathy (structural or cellular changes) in the examined bones or articular cartilages or any effects on bone size or bone mass. Toxicokinetic analysis showed satisfactory systemic exposure [236].

4.8.3.5 LOCAL TOLERABILITY STUDIES

Local tolerability studies were conducted to evaluate the potential local irritant effects of ozenoxacin after dermal and ocular administration to different species (rabbits, mice, guinea pigs).

Skin sensitization resulting in allergic contact dermatitis is the most common manifestation of immunotoxicity in humans. Many hundreds of chemicals have been shown to possess the ability to induce skin sensitization, and allergic contact dermatitis remains an important occupational and environmental health problem [237, 238]. Consequently, it important to evaluate and characterize by appropriate methods the potential risks for human health [239]. Therefore, a specific contact sensitization study was conducted using the local lymph node assay (LLNA) in mice and dermal tolerability were also conducted. The LLNA lymph node assay is a murine model developed to evaluate the skin sensitization potential of chemicals which relies on measurement of events induced during the induction phase of skin sensitization, specifically lymphocyte proliferation in the draining lymph nodes which is a hallmark of a skin sensitization response [240].

As the chemically-related quinolone family is known to be associated with photosensitivity and phototoxicity reaction, mostly caused by the presence of a halogen substituent at 6-position in the quinolone structure. Ozenoxacin is a non-fluorinated quinolone with no halogen substituent at 6-position; consequently, this adverse effect is very unlikely to be produced. However, specific studies were conducted to rule-out the potential production of these effects.

The details of the local-tolerability studies are included in the table below.

Table 24. Local tolerability studies

Objectives	Species	Form	Route	Duration	Ref
Skin irritation	Rabbits	Cream 1%	Dermal (intact & abraded skin), ocular	14 days	[241]
Skin sensitization	Murine LLNA	Cream 0.5%, 1%, 2%	Dermal	3 days	[242]
Phototoxicity	Albino guinea pigs	Ointment 1%	Dermal	Single dose	[243]
Photoallergy, skin sensitization	Albino guinea pigs	Ointment 0.35, 0.75, 1%	Dermal	Single dose (+ induction)	[244]

A repeated-dose local tolerability study was conducted with a 1% cream formulation of ozenoxacin applied dermally (during 14 days to intact and abraded skin) and via ocular (single-dose) to rabbits. ozenoxacin produced mild skin irritation (especially in abraded skin applications) and moderate irritation on ocular mucosa [241].

A murine LLNA was conducted to assess the potential skin of sensitization of different concentration of an ozenoxacin cream formulation. No sensitization was detected in the study [242].

A single-dose study was conducted to evaluate the phototoxic potential of ozenoxacin after topical administrations of different concentrations of an ozenoxacin ointment formulation to albino guinea pigs. No phototoxic effects were detected in the study [243].

A single-dose (after a previous induction administration phase) study was conducted to evaluate the photoallergic and skin sensitization potential of ozenoxacin after topical administrations of different concentrations of an ozenoxacin ointment formulation to albino guinea pigs. No photoallergenic. or skin sensitizing effects were detected in the study [244].

4.8.3.6 Preclinical bioavailability

The absorption from the skin after topical administrations of different ozenoxacin formulations and concentrations has been evaluated in different *in-vivo* preclinical models

such rat and dog and minipig models (minipigs are used because of their similar skin structure to human skin).

The details of the *in-vitro* absorption preclinical studies are included in the table below.

Table 25. *In-vivo* absorption preclinical studies

Species	Form	Strength	Dose	Duration	Ref.
Rat	Ointment	1, 6.3, 40%	o.d.	28 days	[219]
Dog	Ointment	1%	o.d.	1 dose	[245]
Minipigs	Ointment	1, 6.3, 40%	o.d	28 days	[220]
Rat (injured skin)	Gel	1%	t.i.d.	3 days	[246]
Minipigs	Ointment Cream	1, 20%	o.d.	14 days	[247]
Rat	Cream	2% [^{14}C]-ozenoxacin	o.d.	1 dose	[248]
Minipigs	Cream	2% [^{14}C]-ozenoxacin	o.d.	1 dose	[249]
Minipigs (intact and abraded skin)	Cream	1%	o.d.	28 days	[221]

In various *in-vivo* studies conducted with different animal species and topical applications of different formulations and doses, plasma concentrations were generally below the LOQ. Even after dermal application of radiolabeled compound to rat and minipigs, virtually no radioactivity was detected in plasma samples [248, 249]. The very low recovery of radioactivity in feces and urine supports the conclusion that absorption from the skin is extremely low. Only after repeated application (28 days) of very high doses or application on injured skin (rats), measurable concentrations were observed in rats and minipigs. The low permeation through excised human skin observed in vitro (<0.013% for 1% ointment and <0.01% for 1% and 2% creams) and in skin from juvenile Yucatan minipigs (<0.018% for 1% cream) further confirm these findings.

All experiments testing the topical formulation demonstrated an extremely limited absorption of ozenoxacin from the skin. The studies in minipigs probably are the most representative to extrapolate these findings to humans since the skin characteristics of both species are similar.

4.8.4 Clinical data

The clinical development of ozenoxacin cream has included several phase I clinical trials in healthy volunteers, a phase II clinical trial in patients with secondarily infected traumatic lesions (SITLs), a phase III clinical trial in adult and pediatric patients (aged 2 years and older) with impetigo , a second phase III clinical trial in adult and pediatric patients (aged 2 months and older) with impetigo (which has recently been completed) and the present

phase I/II clinical trial in adult and pediatric patients (aged 2 months and older) with impetigo. In all these studies, ozenoxacin has been applied in different concentrations to different extensions of intact and abraded skin and with different frequencies of administration and have allowed to demonstrate the lack of absorption, the good safety and tolerability and the efficacy of the product.

Apart of general guidelines applicable to clinical development and to the conduction of clinical trials, specific guidelines for clinical trials with antimicrobials and for the development of drugs for ABSSSIs have also been taken into account in the design of the clinical development of ozenoxacin [250, 251, 252].

The clinical development plan has been discussed and agreed with different Regulatory Authorities such as US Food and Drug Administration (FDA), European Medicines Agency (EMA) Pediatric Committee (PDCO), Medicines and Healthcare Products Regulatory, UK Medicines and Healthcare Products Regulatory (MHRA), and Spanish Agencia Española de Medicamentos y Productos Sanitarios (AEMPS). The FDA and the PDCO have also agreed to the specific pediatric investigational plan (PIP) for ozenoxacin.

A summary of the clinical trials conducted with ozenoxacin in healthy volunteers is included in the table below.

Table 26. Clinical trials conducted with topical applications of ozenoxacin in healthy volunteers

Phase	Objective	Formulation	N pts (Total)	N pts (Ozn)	Area exposed	N doses	Subjects
Phase I	Absorption, safety, tolerability	Ointment 1%	24	24	9 cm^2 90 cm^2 900 cm^2	1	Adult healthy volunteers
Phase I	Absorption, safety, tolerability	Ointment 1%	20	19	90 cm^2	3/day 7 days	Adult healthy volunteers
Phase I	Absorption, safety, tolerability	Cream 2%	20	20	90 cm^2	2/day 7 days	Adult healthy volunteers
Phase I	Absorption, safety, tolerability (normal and abraded skin)	Cream 2%	24	18	200 cm^2 Abraded skin	2/day 7 days	Adult healthy volunteers
Phase I	Dermal exposure safety, tolerability	Cream 2%	24	24	49 cm^2	1/day, 2/day 3 days	Adult healthy volunteers
Phase I	Cumulative irritation (normal and abraded skin)	Cream 2%	37	37	0.5 cm^2 abraded & 0.5 cm^2 intact skin	21 days/ 1 application per day	Adult healthy volunteers
Phase I	Phototoxicity	Cream 2%	32	32	8 cm^2	1 application / 1 day	Adult healthy volunteers
Phase I	Sensitizing Potential	Cream 2%	200	200	0.5 cm^2	10 applications / 10 days	Adult healthy volunteers

Phase	Objective	Formulation	N pts (Total)	N pts (Ozn)	Area exposed	N doses	Subjects
Phase I	Photoallergy	Cream 2%	60	60	8 cm^2	7	Adult healthy volunteers
Phase I	Cumulative irritation (normal and abraded skin)	Cream 1%	33	33	0.5 cm^2 abraded & 0.5 cm^2 intact skin	21 days/ 1 application per day	Adult healthy volunteers
Phase I	Phototoxicity	Cream 1%	32	32	8 cm^2	11 application / 1 day	Adult healthy volunteers
Phase I	Sensitizing Potential	Cream 1%	220	220	0.5 cm^2	10 applications / 10 days	Adult healthy volunteers
Phase I	Photoallergy	Cream 1%	59	59	8 cm^2	7	Adult healthy volunteers

A summary of the clinical trials conducted with ozenoxacin in patients is included in the tables below.

Table 27. Clinical trials conducted with topical applications of ozenoxacin in patients

Phase	Objective	Formulation	N pts (Total)	N pts (Ozn)	Area exposed	N doses	Subjects
Phase II	Dose finding, efficacy, safety, tolerability (SITLs)	Cream 0.25% Cream 1% Cream 2%	202	151	≤ 100 cm^2	2/day 7 days	Patients with SITLs
Phase I-II	Absorption, safety, tolerability (impetigo)	Cream 1%	46	46	≤ 100 cm^2	2/day 5 days	Patients ≥ 2 months with impetigo
Phase III	Efficacy, safety , tolerability (impetigo)	Cream 1%	465	156	≤ 100 cm^2	2/day 5 days	Patients ≥ 2 years with impetigo
Phase III	Efficacy, safety , tolerability (impetigo)	Cream 1%	412	206	≤ 100 cm^2	2/day 5 days	Patients ≥ 2 months with impetigo

The absorption, safety and tolerability of topical applications of ozenoxacin in humans have been extensively evaluated and characterized in *in-vitro* studies conducted with human skin, in the phase I clinical trials conducted in healthy volunteers and in the phase I-II clinical trial conducted in adult and pediatric patients (aged 2 months and older) with impetigo. The design, methodology and results of these studies have been published and are presented and discussed in the present document.

Additional safety and tolerability data of ozenoxacin is also available from a phase II clinical trial conducted in adult patients with SITLs, and two phase III clinical trials conducted in adult and pediatric patients with impetigo (the first one included patients aged 2 years and older and the second one included patients aged 2 months and older). All these studies have provided data of efficacy, safety, and tolerability in pediatric and adult patients.

The efficacy, safety, and tolerability of three doses of ozenoxacin cream (0.25%, 1% and 2%) and placebo applied b.i.d. during 7 days were evaluated in a phase II dose-finding

clinical trial in 202 adult patients with secondarily infected traumatic lesions (SITLs). The analysis of clinical and microbiological response in the phase II study showed statistically significant superiority of ozenoxacin 1% over placebo at end of treatment. In this study, all there doses were safe and very well tolerated. Few application site effects have been reported (irritation, pruritus) with no differences between the different ozenoxacin doses and placebo [253, 254].

The results of this phase II clinical trial in patients with SITLs were considered to be translatable also to impetigo. Since both SITLs and Impetigo are caused mainly by the same pathogens (*Staphylococcus aureus* and *Streptococcus pyogenes*) [122, 123, 255] in both adult and pediatric populations, the 1% dose administered twice daily was considered adequate for further development in impetigo. Regarding the duration of treatment, considering the potent in vitro activity of ozenoxacin against the pathogens causing impetigo (*Staphylococcus aureus* and *Streptococcus pyogenes*) a 5 day treatment period was considered justifiable for development in impetigo. The formulation (cream), dose (1% ozenoxacin) and posology (twice daily during 5 days) was selected for further development and included in the phase III confirmatory studies in patients with impetigo. According to regulatory requirements, the evaluation of the absorption of an active ingredient after topical applications in the target population needs to be conducted with the final (to be marketed) formulation. Therefore the same formulation (cream), dose (1% ozenoxacin) and posology (twice daily during 5 days) was evaluated in the phase I-II absorption clinical trial conducted in patients with impetigo

A phase III clinical study to compare the efficacy and safety of a b.i.d. topical application for 5 days (10 applications) of ozenoxacin 1% cream versus placebo in 465 adult and pediatric patients (aged 2 years old and older) with impetigo, has also been conducted. In this study, ozenoxacin was statistically superior to placebo in both clinical and microbiological success rates with similar clinical success rates than retapamulin. However, ozenoxacin produced a more rapid microbiological clearance of the impetigo lesions than retapamulin. . In this study, ozenoxacin was statistically superior to placebo in both clinical and microbiological success rates with similar clinical success rates than retapamulin. [256, 257, 258].

A second phase III study to compare the efficacy and safety of a b.i.d. topical application for 5 days (10 applications) of ozenoxacin 1% cream versus placebo in 412 adult and pediatric patients (aged 2 months and older) with impetigo has recently been completed. [259, 260]. In this study, ozenoxacin was statistically superior to placebo in both clinical and microbiological success rates with similar clinical success rates than retapamulin.

The clinical development conducted so far has extensively characterized the safety, tolerability, and efficacy of ozenoxacin in adult and pediatric population and has allowed the selection of the optimal topical formulation, dose, and posology of ozenoxacin for final

development and marketing. The data generated during the clinical development together with the preclinical data will serve as the basis of regulatory applications for marketing approval.

5 ABSORPTION, SAFETY AND TOLERABILITY OF OZENOXACIN

5.1 JUSTIFICATION

Topical antibacterial agents are valuable tools in antimicrobial therapy for both inpatient and outpatient treatment and available clinical evidence supports the use of topical agents for treatment of uncomplicated SSSIs. Most simple uncomplicated skin infections do not require treatment with systemic antibiotics but do benefit from the use of topical antimicrobial agents. Topical antibiotics offer a useful alternative to oral and parenteral agents as they are easy to use, are equally as effective as systemic antibiotics, cause fewer side effects than systemic preparations, and result in higher drug concentrations at the infected area. Although different therapeutic options exist for the treatment of SSSIs, the increasing incidence of resistant strains of the bacteria causing these infections (e.g. community acquired methicillin-resistant *S. aureus*) justifies the need to develop alternative antibiotics to treat impetigo.

Ozenoxacin is a non-fluorinated quinolone formulated as a 1% cream for topical use which is currently in development for the topical treatment of various uncomplicated skin and skin structure infections including impetigo.

The preclinical studies conducted with ozenoxacin have demonstrated:

- A potent bactericidal effect against pathologically relevant Gram-positive bacteria, especially *Staphylococci* and *Streptococci* strains (including strains resistant to other antibiotics) which are usually involved in ABSSSIs. An excellent activity has been demonstrated against *S. aureus* with double or triple mutations in the QRDR of gyrase A and topoisomerase IV genes, confirming the dual target mechanism of action. *In-vivo* activity in murine experimental models of dermal infection by *S. aureus* has also been demonstrated.
- A broad safety margin. Adverse effects usually related to fluorinated quinolones, such as phototoxicity, photoallergenic, sensitizer potential, mutagenicity/genotoxicity and articular toxicity have not been observed in any of the preclinical studies conducted. A very good tolerability profile has even been demonstrated in extreme conditions such a study conducted in abraded skin in mini-pigs after daily dermal administrations during 28 consecutive day's period. Furthermore, in specific studies conducted in animal models no evidences of chondrotoxicity were found, thus reinforcing the excellent safety profile of ozenoxacin. The excellent safety profile demonstrated during the preclinical development supported the initiation of the clinical development of the compound and its administration to humans.
- A low potential of absorption in topical applications in *in-vitro* and *in-vivo* studies.

This good safety profile has allowed the conduction a clinical development program which has shown a lack of absorption, a very good safety and tolerability profile, and provided evidence of the clinical and bacteriological efficacy of the product.

The clinical development plan of ozenoxacin included the following studies:

- Five (5) *in-vitro* studies to evaluate the percutaneous absorption (4 studies) and skin metabolism (1 study) of different ozenoxacin formulations applied to human skin samples.
- Four (4) phase I clinical trials (conducted in adult healthy volunteers) to evaluate the absorption, safety and the tolerability of different ozenoxacin topical formulations, administration regimens, and doses applied to different skin extensions under normal and extreme conditions
- One (1) Phase I clinical trial to evaluate the dermal exposure, safety, tolerability of two topical administration regimens (once daily and twice daily) of ozenoxacin 2% cream.
- Eight (8) phase I clinical trials (conducted in healthy volunteers) to evaluate the dermal tolerability of different ozenoxacin cream doses (concentrations).
- One (1) phase II clinical trial (conducted in adult patient with SITLs) to evaluate the most effective dose of ozenoxacin cream.
- Two (2) phase III clinical trials conducted in pediatric and adult patients with impetigo to evaluate the efficacy, safety, and tolerability of ozenoxacin 1% cream. One of the phase III clinical trials has been conducted in adult and pediatric patients (aged 2 years and older) and the other phase III clinical trial has been conducted in adult and pediatric patients (aged 2 months and older).
- One (1) phase I-II clinical trial (conducted in adult pediatric (aged 2 months and older) patients with impetigo) to evaluate the absorption, safety, tolerability and clinical effect.

This clinical development plan has been thoroughly discussed and agreed with following Regulatory Authorities:

- US Food and Drug Administration (FDA).
- European Medicines Agency (EMA).
- EMA Pediatric Committee (PDCO).
- UK Medicines and Healthcare Products Regulatory Agency (MHRA).
- Spanish Agencia Española de Medicamentos y Productos Sanitarios (AEMPS).

The FDA and the PDCO have also agreed to the specific pediatric investigational plan (PIP) for ozenoxacin. In the case of ozenoxacin, the pediatric clinical development plan

consisted in the inclusion of children of different age subsets in the phase III clinical trials and in the phase I-II clinical trial all conducted in patients with impetigo.

An important consideration for the development of products intended for pediatric administration is that a very good evaluation of the safety and tolerability should be first conducted in adults before the administration in children. Additionally, the development of a topical compound should include as part of the safety characterization, an evaluation of the systemic drug exposure following the application of the topical agent to healthy volunteers and to patients with the target indication. This is particularly important in infants, who have a significantly higher ratio of body surface area to body mass than older children and adults.

As one the intended indications of the development of a topical formulation of ozenoxacin is impetigo which affects mainly the pediatric population, it was very important to characterize the absorption, safety and tolerability for the potential use in this population. The microbiological profile shown by ozenoxacin, makes it a good candidate for use in this indication. Despite the very good safety, tolerability and toxicological profile shown in during the preclinical development, taking into account the possible risks related to quinolones (specially, in children), it was considered very important to show a very good safety and tolerability profile and lack of absorption or negligible absorption in clinical studies.

The phase I clinical trials conducted in adult healthy volunteers have allowed to characterize the potential absorption, and the overall safety and tolerability as well as the dermal tolerability (irritation, sensitization, phototoxicity and photoallergy) of different topical formulations, concentrations and administration regimens applied to different skin extensions, under normal and extreme conditions (e.g. high concentrations, large skin extensions, and abraded skin). One phase I clinical trial was conducted to characterize the skin exposure/penetration of ozenoxacin in the different skin layers. These clinical trials; together with the results of a phase II clinical trial conducted in adults with SITLs and studies conducted in *in-vitro* models of human skin, have allowed to select the more appropriate dose, formulation, and posology which has been tested in phase III clinical trials. A phase I-II clinical trial was also conducted with the finally selected (to be marketed) formulation (ozenoxacin 1% cream) in order to evaluate absorption, safety, tolerability and initial efficacy in adult and pediatric patients (aged 2 months to 18 years old) with the target disease (impetigo).

The aim of these studies has been to address a number of important questions regarding the pharmacokinetic profile (absorption and dermal exposure), safety and tolerability; including specific studies to evaluate the dermal tolerability (irritation, sensitization, phototoxicity and photoallergy) of ozenoxacin. Most of these studies have been conducted in adult healthy volunteers. But the data is also complemented by *in-vitro* studies

conducted with human skin samples and by a phase I-II clinical trial designed to evaluate the absorption, safety, and tolerability of ozenoxacin in adult and pediatric population with impetigo (including different subsets of pediatric patients).

All these studies have contributed to define the absorption, safety and tolerability profile in adult healthy volunteers in adult and pediatric patients with impetigo and have allowed to select the optimal formulation for full development and marketing (ozenoxacin 1% cream) and the best posology (twice daily applications during 5 days) in this indication. The safety and tolerability profile of these studies has also been completed by the data from the mentioned phase II clinical trial conducted in adult patients with impetigo and by the phase two III clinical trials conducted in adult and pediatric patients (the first one included patients aged 2 years and older and the second one included patients aged 2 months and older) with impetigo.

The present work show the different studies conducted as part of the clinical development plan of ozenoxacin focusing in the studies conducted to specifically evaluate and characterize its absorption, safety and tolerability and include the following studies (the phase II and III clinical trials which were conducted to evaluate the efficacy, safety, and tolerability of ozenoxacin are not included as part of the present work; although, the safety and tolerability results are commented when applicable).

- **In-vitro percutaneous absorption and metabolism studies:** 5 *in-vitro* studies to evaluate the percutaneous absorption (4 studies) and skin metabolism (1 study) of different ozenoxacin formulations applied to human skin samples.
- **Systemic bioavailability, safety and tolerability studies:** 4 phase I clinical trials (conducted in adult healthy volunteers) to evaluate the absorption, safety and the tolerability of different ozenoxacin topical formulations, administration regimens, and doses applied to different skin extensions under normal and extreme conditions.
- **Skin tissue exposure study:** 1 phase I clinical trial to evaluate the dermal exposure, safety, tolerability of two topical administration regimens (once daily and twice daily) of ozenoxacin 2% cream.
- **Dermal tolerability studies:** 8 phase I clinical trials (conducted in healthy volunteers) to evaluate the dermal tolerability of different ozenoxacin cream doses (concentrations).
- **Systemic bioavailability and safety in impetigo study:** 1 phase I-II clinical trial (conducted in adult pediatric (aged 2 months and older) patients with impetigo) to evaluate the absorption, safety, tolerability and clinical effect.

The design, methodology, and results have been described in different publications and are presented and commented as part of the present work in the sections below.

5.2 General hypotheses

Taking into account the excellent ozenoxacin absorption, safety, tolerability and toxicological profile shown during the preclinical development it was expected that:

- Ozenoxacin would show a lack of absorption or negligible absorption in clinical studies after application of different topical formulations, skin extensions, administration regimens, and concentrations applied under normal and extreme conditions to human skin and healthy volunteers.
- Ozenoxacin would show a very good safety and tolerability profile after application of different topical formulations, skin extensions, administration regimens, and concentrations applied under normal and extreme conditions to healthy volunteers.
- Ozenoxacin would show a lack of absorption or negligible absorption and a very good safety and tolerability profile after topical applications to adult and pediatric patients (in different age subsets starting with 2 months old) with impetigo thus showing the reproducibility of the results obtained in healthy volunteers in the intended indication.
- These results should server to support the favorable benefit-risk relationship for a product for its use in a predominantly pediatric indication.

5.3 STUDY SPECIFIC HYPOTHESES

5.3.1 IN-VITRO PERCUTANEOUS ABSORPTION AND METABOLISM

Taking into account different absorption studies conducted during the preclinical development, it was expected that no permeation or low permeation will be detected after application of ozenoxacin formulations and concentrations to human skin in an *in-vitro* Franz cell model (as previously observed in preclinical *in-vivo* and *in-vitro* studies).

It was expected that no difference will be observed in the permeation of ozenoxacin after administration of a 1% cream to samples of human skin of different ethnic origin.

No skin metabolism was expected after application of ozenoxacin to samples of human skin in an *in-vitro* model.

5.3.2 SYSTEMIC BIOAVAILABILITY, SAFETY AND TOLERABILITY

A low systemic absorption was expected to be shown after single and repeated applications after application of different ozenoxacin formulations (ointment and cream), in different strengths (1% and 2%) to different body surface areas (from 90 to 900 cm2), to intact and abraded skin in healthy volunteers.

Ozenoxacin was expected to show a very good safety and tolerability profile after single and repeated applications after application of different ozenoxacin formulations (ointment and cream), in different strengths (1% and 2%) to different body surface areas (from 90 to 900 cm2), to intact and abraded skin in healthy volunteers.

5.3.3 SKIN TISSUE EXPOSURE

Based on previous *in-vitro* studies and absorption studies conducted in healthy volunteers, it was expected that higher concentrations would be found in the outer layers of the skin after topical applications of ozenoxacin cream.

Twice daily topical applications of ozenoxacin cream should provide higher concentrations in the skin than once daily applications.

Ozenoxacin will show a very good safety and tolerability profile after once daily and twice daily applications.

5.3.4 DERMAL TOLERABILITY STUDIES

Dermal tolerability studies conducted during the preclinical development of ozenoxacin have shown not relevant irritation, sensitizing, phototoxicity and photoallergy potential effects. No clinically relevant dermal reactions were found in the different phase I clinical trials conducted in healthy adult volunteers.

Therefore, no dermal tolerability effects (cumulative irritation, sensitizing, phototoxicity and photoallergy potential) expected after applications of ozenoxacin 1% and 2% cream formulations in healthy adult volunteers.

5.3.5 SYSTEMIC BIOAVAILABILITY AND SAFETY IN IMPETIGO

Clinical trials conducted in healthy adult volunteers, showed no systemic absorption together with a very good safety and tolerability profile of ozenoxacin. Similar results (no absorption or low absorption, and good safety and tolerability profile) were expected in adults and pediatric patients of different age groups (aged 2 months and older) with the target indication (impetigo).

Based on the results of preclinical studies and clinical trials in healthy volunteers, the systemic absorption of ozenoxacin was not expected to be higher in the pediatric population than in adults with mature skin.

These results should server to further support the favorable benefit-risk relationship for a product for its use in a predominantly pediatric indication.

5.4 General objectives

To evaluate the absorption, safety and tolerability of ozenoxacin after application of different topical formulations, skin extensions, administration regimens, and concentrations applied under normal and extreme conditions to human skin and healthy volunteers.

To evaluate the absorption, safety and tolerability of ozenoxacin after topical applications to adult and pediatric patients (aged 2 months old and older) with impetigo.

5.5 STUDY SPECIFIC OBJECTIVES

5.5.1 IN-VITRO PERCUTANEOUS ABSORPTION AND METABOLISM

The main objectives of these studies were:

- To evaluate the percutaneous absorption of ozenoxacin from different ozenoxacin topical formulations in *in-vitro* models of human skin.
- To evaluate possible differences in percutaneous of an ozenoxacin topical formulation in human skin of different ethnic origins in an *in-vitro* model of human skin.
- To evaluate the skin metabolism of ozenoxacin after application of a topical formulation of ozenoxacin.

5.5.2 SYSTEMIC BIOAVAILABILITY, SAFETY AND TOLERABILITY

To evaluate the systemic bioavailability, safety and tolerability of different topical formulations, skin extensions, administration regimens, and concentrations applied under normal and extreme conditions in healthy volunteers.

5.5.3 SKIN TISSUE EXPOSURE

To evaluate the kin tissue exposure of once-daily versus twice-daily topical applications of ozenoxacin to healthy volunteers.

To evaluate the safety and tolerability of once daily and twice daily topical applications of ozenoxacin to healthy volunteers.

5.5.4 DERMAL TOLERABILITY STUDIES

To evaluate the dermal tolerability (cumulative irritation, sensitizing, phototoxicity and photoallergy potential) of ozenoxacin 1% and 2% cream formulations in healthy adult volunteers.

5.5.5 SYSTEMIC BIOAVAILABILITY AND SAFETY IN IMPETIGO

To evaluate the systemic bioavailability of twice-daily topical ozenoxacin 1% cream in adults and children with impetigo.

To evaluate the safety, tolerability of twice-daily topical ozenoxacin 1% cream in adults and children with impetigo.

To evaluate the initial clinical response of twice-daily topical ozenoxacin 1% cream in adults and children with impetigo.

5.6 Materials, Methods, and Results

The materials, methods, and results of the studies mentioned above are detailed in the 5 different publications included below.

5.6.1 In-vitro percutaneous absorption and metabolism

As part of the clinical development of a topical formulation of ozenoxacin, various in-vitro studies were conducted to evaluate the percutaneous absorption and the metabolism of ozenoxacin after applications of different topical ozenoxacin formulations (ointment and cream). Five of these studies were conducted in excised human skin and included four (4) Franz Cell percutaneous absorption studies and one (1) skin metabolism study as indicated below:

- *In-vitro* percutaneous absorption of ozenoxacin ointment 1% through human skin (P-040127-01) [261].
- *In-vitro* percutaneous absorption of dermal formulations of ozenoxacin (1% ointment or 1% cream) through human skin (P-080567-01) [262].
- *In-vitro* percutaneous absorption of dermal formulations of ozenoxacin (1% ointment or 2% cream) through human skin (P-080632-01) [263].
- *In-vitro* percutaneous absorption of ozenoxacin 1% cream through human skin from different ethnic origins (P-120931-01) [264].
- *In-vitro* skin metabolism study P-080655-01 [265].

The materials, methods, and results are described in a publication included below [266].

Santos B, Ortiz J, Gropper S. In vitro percutaneous absorption and metabolism of ozenoxacin in excised human skin. Future Microbiol. 2014;9 (Sep) (8 Suppl):S3-9.

Santos B, Ortiz J, Gropper S. In vitro percutaneous absorption and metabolism of ozenoxacin in excised human skin. Future Microbiol. 2014;9 (Sep) (8 Suppl):S3-9.

Santos B, Ortiz J, Gropper S. In vitro percutaneous absorption and metabolism of ozenoxacin in excised human skin. Future Microbiol. 2014;9 (Sep) (8 Suppl):S3-9.

Santos B, Ortiz J, Gropper S. In vitro percutaneous absorption and metabolism of ozenoxacin in excised human skin. Future Microbiol. 2014;9 (Sep) (8 Suppl):S3-9.

Santos B, Ortiz J, Gropper S. In vitro percutaneous absorption and metabolism of ozenoxacin in excised human skin. Future Microbiol. 2014;9 (Sep) (8 Suppl):S3-9.

Santos B, Ortiz J, Gropper S. In vitro percutaneous absorption and metabolism of ozenoxacin in excised human skin. Future Microbiol. 2014;9 (Sep) (8 Suppl):S3-9.

Santos B, Ortiz J, Gropper S. In vitro percutaneous absorption and metabolism of ozenoxacin in excised human skin. Future Microbiol. 2014;9 (Sep) (8 Suppl):S3-9.

5.6.2 SYSTEMIC BIOAVAILABILITY, SAFETY AND TOLERABILITY

As part of the clinical development of a topical formulation of ozenoxacin, various of phase I clinical trials were conducted in adult healthy volunteers to examine the systemic bioavailability, safety, and tolerability of different ozenoxacin formulations. These studies included different ozenoxacin formulations (ointment and cream), different strengths (1% and 2%) applied to different body surface areas (from 90 to 900 cm2), in intact and abraded skin and single and repeated applications. Four studies were conducted as indicated below:

- GF-001001-00 ointment formulation. A phase I, open label, single-raising dose, parallel group study to examine the systemic bioavailability of topical applications to healthy volunteers (P-050374-01) [267].
- GF-001001-00 ointment formulation. A phase I, single-blind, 2-way cross-over, placebo controlled, multiple dose study to examine the systemic bioavailability of topical applications to healthy volunteers (P-070515-01) [268].
- GF-001001-00 cream formulation. A phase I, single-blind, 2-way cross-over, placebo controlled, multiple dose study to examine the systemic bioavailability of topical applications to healthy volunteers (P-080582-01) [269].
- GF-001001-00. A phase I, single-blind, cross-over, randomized study to examine the systemic bioavailability of topical applications of 2% cream on intact and abraded skin in healthy volunteers (P-090778-01) [270].

A summary of these phase I clinical trials (including type of formulation, strength, number of subjects exposed, approximate dose applied per application, posology per day, and days of application) is included in the table below.

Table 28. Phase I bioavailability, safety and tolerability clinical trials of topical applications of ozenoxacin conducted in adult healthy volunteers

Study code	Skin	Form	Strength	N subjects (Total)	Area exposed	Dose (approx) per application (g)	Dose x day	Ref.
P-050374-01	Normal	Ointment	1%	24	9 cm^2 90 cm^2 900 cm^2	0.05 0.5 5	Single dose Single dose Single dose	[267]
P-070515-01	Normal	Ointment	1%	19	90 cm^2	0.5	t.i.d 7 days	[268]
P-080582-01	Normal	Cream	2%	20	90 cm^2	0.5	b.i.d. 7 days	[269]
P-090778-01	Normal and abraded	Cream	2%	18	200 cm^2	1	b.i.d. 7 days	[270]

The materials, methods, and results are described in a publication included below [271].

Gropper S, Albareda N, Santos B, Febbraro S. Systemic bioavailability, safety and tolerability of topical ozenoxacin in healthy adult volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S11-16.

Gropper S, Albareda N, Santos B, Febbraro S. Systemic bioavailability, safety and tolerability of topical ozenoxacin in healthy adult volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S11-16.

Gropper S, Albareda N, Santos B, Febbraro S. Systemic bioavailability, safety and tolerability of topical ozenoxacin in healthy adult volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S11-16.

Gropper S, Albareda N, Santos B, Febbraro S. Systemic bioavailability, safety and tolerability of topical ozenoxacin in healthy adult volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S11-16.

Gropper S, Albareda N, Santos B, Febbraro S. Systemic bioavailability, safety and tolerability of topical ozenoxacin in healthy adult volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S11-16.

Gropper S, Albareda N, Santos B, Febbraro S. Systemic bioavailability, safety and tolerability of topical ozenoxacin in healthy adult volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S11-16.

5.6.3 SKIN TISSUE EXPOSURE

As part of the development of a topical ozenoxacin formulation, a phase I study was conducted in healthy volunteers with the aim of assessing and compare the skin tissue exposure, safety and tolerability of once- versus twice-daily topical application of ozenoxacin 2% cream [272]. The aim was to provide data on skin exposure for the selection of an optimal posology of the product. A 2% cream was included in the study as it was the highest strength of the formulation initially selected for further clinical development. The safety and tolerability were also assessed.

A description of the total exposure to ozenoxacin of the patients included in this study is included in the table below.

Table 29. Exposure to ozenoxacin in healthy volunteers (skin tissue exposure study)

Formulation	Strength	Dosage	Day	Dose (g)*	Total Dose (g)*	Extension	Subjects	Ref
Cream	2%	1/day	1	0.6	1.2	147 cm^2	12	[272]
			2	0.4		98 cm^2		
			3	0.2		49 cm^2		
	2%	2/day	1	1.2	2.4	147 cm^2	12	
			2	0.8		98 cm^2		
			3	0.4		49 cm^2		
*Approximate dose of formulation								

The materials, methods, and results are described in a publication included below [273].

Gropper S, Albareda N, Santos B, Febbraro S. Skin tissue exposure of once- versus twice-daily topical ozenoxacin 2% cream: a Phase I study in healthy volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S17-22.

Gropper S, Albareda N, Santos B, Febbraro S. Skin tissue exposure of once- versus twice-daily topical ozenoxacin 2% cream: a Phase I study in healthy volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S17-22.

Gropper S, Albareda N, Santos B, Febbraro S. Skin tissue exposure of once- versus twice-daily topical ozenoxacin 2% cream: a Phase I study in healthy volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S17-22.

Gropper S, Albareda N, Santos B, Febbraro S. Skin tissue exposure of once- versus twice-daily topical ozenoxacin 2% cream: a Phase I study in healthy volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S17-22.

Gropper S, Albareda N, Santos B, Febbraro S. Skin tissue exposure of once- versus twice-daily topical ozenoxacin 2% cream: a Phase I study in healthy volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S17-22.

Gropper S, Albareda N, Santos B, Febbraro S. Skin tissue exposure of once- versus twice-daily topical ozenoxacin 2% cream: a Phase I study in healthy volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S17-22.

5.6.4 Dermal tolerability studies

As part of the clinical development of a topical formulation of ozenoxacin, dermal tolerability clinical trials needed to be conducted. These studies were initially conducted with a 2% cream but after obtaining the results of the phase II clinical trial in patients with SITLs, the 1% cream formulation was selected for further development and the dermal tolerability studies were repeated with this last formulation to comply with regulatory requirements.

The following dermal tolerability clinical trials were conducted with ozenoxacin:

- Cumulative irritation study (in normal and abraded skin) with the 2% cream (P-090736-01) [274].
- Phototoxicity study with the 2% cream (P-090737-01) [275].
- Sensitizing Potential with the 2% cream (P-090738-01) [276].
- Photoallergy study with the 2% cream (P-090739-01) [277].
- Cumulative irritation study (in normal and abraded skin) with the 1% cream (P-100845-01) [278].
- Phototoxicity study with the 1% cream P-100846-01 () [279].
- Sensitizing Potential with the 1% cream (P-100847-01) [280].
- Photoallergy study with the 1% cream (P-100848-01) [281].

The materials, methods, and results are described in a publication included below [282].

Gropper S, Cepero AL, Dosik JS, LaStella P, Siemetzki H, Wigger-Alberti W. Cumulative irritation, sensitizing potential, phototoxicity and photoallergy of ozenoxacin in healthy adult volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S23-31.

Gropper S, Cepero AL, Dosik JS, LaStella P, Siemetzki H, Wigger-Alberti W. Cumulative irritation, sensitizing potential, phototoxicity and photoallergy of ozenoxacin in healthy adult volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S23-31.

Gropper S, Cepero AL, Dosik JS, LaStella P, Siemetzki H, Wigger-Alberti W. Cumulative irritation, sensitizing potential, phototoxicity and photoallergy of ozenoxacin in healthy adult volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S23-31.

Gropper S, Cepero AL, Dosik JS, LaStella P, Siemetzki H, Wigger-Alberti W. Cumulative irritation, sensitizing potential, phototoxicity and photoallergy of ozenoxacin in healthy adult volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S23-31.

Gropper S, Cepero AL, Dosik JS, LaStella P, Siemetzki H, Wigger-Alberti W. Cumulative irritation, sensitizing potential, phototoxicity and photoallergy of ozenoxacin in healthy adult volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S23-31.

Gropper S, Cepero AL, Dosik JS, LaStella P, Siemetzki H, Wigger-Alberti W. Cumulative irritation, sensitizing potential, phototoxicity and photoallergy of ozenoxacin in healthy adult volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S23-31.

Gropper S, Cepero AL, Dosik JS, LaStella P, Siemetzki H, Wigger-Alberti W. Cumulative irritation, sensitizing potential, phototoxicity and photoallergy of ozenoxacin in healthy adult volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S23-31.

Gropper S, Cepero AL, Dosik JS, LaStella P, Siemetzki H, Wigger-Alberti W. Cumulative irritation, sensitizing potential, phototoxicity and photoallergy of ozenoxacin in healthy adult volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S23-31.

Gropper S, Cepero AL, Dosik JS, LaStella P, Siemetzki H, Wigger-Alberti W. Cumulative irritation, sensitizing potential, phototoxicity and photoallergy of ozenoxacin in healthy adult volunteers. Future Microbiol. 2014;9 (Sep) (8 Suppl):S23-31.

5.6.5 Systemic bioavailability and safety in impetigo

As part of the clinical development of ozenoxacin, a study was conducted to evaluate the absorption, safety, and tolerability, and initial clinical response of ozenoxacin 1% cream in adult and pediatric patients with impetigo [283].

The materials, methods, and results are described in a publication included below [284].

Gropper S, Cepero AL, Santos B, Kruger D. Systemic bioavailability and safety of twice-daily topical ozenoxacin 1% cream in adults and children with impetigo. Future Microbiol. 2014;9 (Sep) (8 Suppl):S33-40.

Gropper S, Cepero AL, Santos B, Kruger D. Systemic bioavailability and safety of twice-daily topical ozenoxacin 1% cream in adults and children with impetigo. Future Microbiol. 2014;9 (Sep) (8 Suppl):S33-40.

Gropper S, Cepero AL, Santos B, Kruger D. Systemic bioavailability and safety of twice-daily topical ozenoxacin 1% cream in adults and children with impetigo. Future Microbiol. 2014;9 (Sep) (8 Suppl):S33-40.

Gropper S, Cepero AL, Santos B, Kruger D. Systemic bioavailability and safety of twice-daily topical ozenoxacin 1% cream in adults and children with impetigo. Future Microbiol. 2014;9 (Sep) (8 Suppl):S33-40.

Gropper S, Cepero AL, Santos B, Kruger D. Systemic bioavailability and safety of twice-daily topical ozenoxacin 1% cream in adults and children with impetigo. Future Microbiol. 2014;9 (Sep) (8 Suppl):S33-40.

Gropper S, Cepero AL, Santos B, Kruger D. Systemic bioavailability and safety of twice-daily topical ozenoxacin 1% cream in adults and children with impetigo. Future Microbiol. 2014;9 (Sep) (8 Suppl):S33-40.

Gropper S, Cepero AL, Santos B, Kruger D. Systemic bioavailability and safety of twice-daily topical ozenoxacin 1% cream in adults and children with impetigo. Future Microbiol. 2014;9 (Sep) (8 Suppl):S33-40.

Gropper S, Cepero AL, Santos B, Kruger D. Systemic bioavailability and safety of twice-daily topical ozenoxacin 1% cream in adults and children with impetigo. Future Microbiol. 2014;9 (Sep) (8 Suppl):S33-40.

5.7 DISCUSSION

Providing to children a better access to safe and effective medications has become a major priority of many countries in the last decade. In fact, this has been reflected in different initiatives mainly by the WHO, the EU, the EMA, and the FDA and has been reflected in different guidelines and recommendations for drug development. As part of these initiatives and regulations, the clinical development of a new drug should include the evaluation of the pharmacokinetics, the efficacy, the safety, and the tolerability of the product not only in adults but also in pediatric population. The development of a topical formulation to treat a dermal condition should also include an evaluation of the absorption after application of the product to the skin. As the objective in this case is usually local action, a low systemic absorption is ideally desired while maintaining an adequate concentration at the site of action.

Ozenoxacin has demonstrated a potent bactericidal effect against pathologically relevant Gram-positive bacteria responsible for ABSSSIs, especially *Staphylococci* and *Streptococci* strains (including strains resistant to other antibiotics) and therefore is a good candidate to treat these infections; especially impetigo.

As impetigo is an infection affecting predominantly the pediatric population with higher incidence in young children (2-6 years of age), the absorption, safety, and tolerability of ozenoxacin needed to be evaluated thoroughly; specially taking into account the safety concerns raised for the use of quinolones in children. Because of the characteristics of impetigo affecting mostly the pediatric population, a joined adult and pediatric development plan was conducted with special focus on safety and tolerability in the pediatric population.

As ozenoxacin is a non-fluorinated quinolone, no phototoxic or photoallergic reactions usually associated with fluoroquinolones were expected; this has been confirmed in preclinical studies. Preclinical studies conducted in juvenile animals specifically designed to evaluate the potential damage to the musculoskeletal system found no damage to the cartilage, and or tendons. Additionally, ozenoxacin showed a good safety and tolerability profile in all the preclinical toxicology studies showed. These findings provided safety assurance for the potential use of the product in children that needed confirmation in clinical studies.

As commented previously, the present work includes the different studies conducted as part of the clinical development plan of ozenoxacin focusing in the studies conducted to specifically evaluate and characterize its absorption, safety and tolerability and include included *in-vitro* studies with human skin, absorption, safety, and tolerability clinical trials in healthy volunteers, a dermal exposure study in healthy volunteers, dermal tolerability studies in healthy volunteers and an absorption, safety, and tolerability clinical trial conducted in patiens with impetigo (which included pediatric and adult population).

The design, methodology and results of these studies have been included in different publications which include the correspondent discussions and conclusions. Additional considerations and complementary discussion is included below.

Four percutaneous absorption studies and one skin metabolism study were performed in *in-vitro*models with human skin. *In-vitro* studies using Franz diffusion cells are usually conducted as part of the development of topical formulations in order to evaluate the percutaneous absorption and the metabolism of the active ingredient contained in these formulations. The Franz Cell is an in-vitro skin permeation assay that consists of two chambers separated by a membrane. It is frequently used in formulation research and development to assess skin permeability and it has evolved into a major research methodology, providing key insights into the relationships between skin, drug and formulation [285, 286]. Different types of membranes can be used (animal, human, synthetic). Although usually difficult to obtain, the use of human skin as membrane in Franz cells studies are preferred in the development of topical drugs for human administration as they provide more predictable results of the clinical application than other type of membranes [287].

The tested product/formulation is applied to the membrane via the upper chamber. The bottom chamber contains fluid from which samples are taken at regular intervals to analyze the amount of active ingredient that has permeated the membrane from the tested formulation at each time point. The chamber is maintained at a constant temperature of (usually 37° C). Depending on the vehicle, the rate of permeation for a given drug as determined via Franz cell analysis can vary significantly. The Franz Cell methodology allows determining whether a particular formulation delivers an active agent through the skin, predicting the in vivo percutaneous absorption of chemicals through the skin, and also allows the evaluation of potential permeability differences between different types of skins [288].

One of the factors to control in Franz Cell studies is the trans-epidermal water loss (TEWL). TEWL is a measure of the flux density of condensed water diffusing from the deeper highly hydrated layers of the dermis and epidermis to the skin surface and is usually expressed in g/m2/h. TEWL values are affected by the state and function of the stratum corneum. Increased TEWL is associated with skin barrier dysfunction whereas normal or decreased TEWL is regarded as an indicator for intact or recovered skin barrier function [289, 290]. TEWL is affected by the anatomic sites, age, climate conditions, and measurement devices among other factors [291, 292]. For standard Franz Cell studies with intact human skin, skin samples with TEWL values above 15 g/m2/h are excluded from the study due to possible defects in the integrity of the stratum corneum.

As already mentioned above, the 4 *in-vitro* Franz-Cell studies and the *in-vitro* skin metabolism study were conducted as part of the development of a topical formulation of

ozenoxacin. The data generated from these studies complemented the results from previous preclinical studies and together with the phase I and phase II clinical trials allowed to select the 1% ozenoxacin cream as the most appropriate topical formulation and strength of ozenoxacin for further development and marketing.

The *in-vitro* percutaneous absorption of ozenoxacin 1% cream through human skin from different ethnic origins was conducted as a complement to the absorption clinical trial conducted in adult and pediatric patients with impetigo [283, 284]. This study had a predominance of patients of black and mixed (multiracial) race, because of the geographical region in which it was conducted. This Franz cell study was therefore conducted in human skin samples from African and Caucasian subjects to evaluate if the skin of black race can present differences in absorption compared to white (Caucasian) skin. The analytical data from this study showed that the levels of ozenoxacin in all the samples from receptor fluid of skin exposed to 1% ozenoxacin cream were below the limit of quantification of this method (0.04 µg/mL) with the exception of two samples for one of the black skin samples that showed values close to the limit of quantification (0.04 µg/mL a 1h and 0.064 µg/mL at 2 h). The highest value detected (0.06 µg/mL) corresponds to less than 0.015% of the applied dose of ozenoxacin onto the skin. These data are similar to the previous Franz cell studies conducted with human skin samples [261, 262, 263]. Therefore, the results of the study seem to indicate that there is no influence of the skin ethnic origin on systemic absorption of ozenoxacin.

In a specific *in-vitro* study, ozenoxacin was found to be metabolically stable in human skin at different concentrations with no metabolites detected, thus assuring the presence of the active unchanged compound in the site of administration where the action is needed.

Four phase I clinical trials to evaluate the bioavailability, safety and tolerability of topical applications of ozenoxacin were conducted in adult healthy volunteers including different ozenoxacin formulations (ointment and cream), different strengths (1% and 2%) applied to different body surface areas (from 90 to 900 cm2), in intact and abraded skin and single and repeated application.

In all these clinical trials of dermal application, the plasma concentration of ozenoxacin was consistently below the limit of quantitation (LOQ), therefore it is concluded that no systemic absorption is observed. This is consistent with the results of non-clinical (preclinical) studies, which showed that the systemic absorption of ozenoxacin is extremely limited when applied topically and that both ointment and cream demonstrates excellent dermal tolerance [219, 220, 221, 245, 246, 247, 248, 249]. The results are also consistent with the results found in Franz cell studies conducted in human skin presented above [261, 262, 263, 264, 266].

No systemic absorption was observed even in extended applications such as 5 g application to 900 cm2 (5% of BSA) or very extreme situations like repeated applications to a surface

of 200 cm^2 of abraded skin. These conditions are considered more extreme than the ones presented the intended indication of impetigo and for the maximum intended topical application (100 cm^2).

All these studies were discussed with different regulatory agencies (US FDA, UK MHRA, Spanish AEMPS, EMA PDCO) and it was considered that the design and results of these studies provided evidence of the safety and tolerability of the product for further development and potential use in pediatric indications.

The bioanalysis of the blood samples was performed with a validated liquid chromatography-tandem mass spectrometry (LS-MS/MS) bioanalytic method with a LOQ of 0.5 ng/mL [293, 294]. This level of sensitivity was considered sensible enough and adequate to conclude no-absorption or negligible and was discussed and accepted by different regulatory agencies (US FDA, UK MHRA, Spanish AEMPS, EMA PDCO). Therefore, it was considered that any potential level below this LOQ would have no potential harmful effects; especially considering the target population for the treatment of impetigo will be mainly children.

Ozenoxacin was shown to be safe and well tolerated after single and repeated applications after application of different ozenoxacin formulations (ointment and cream), in different strengths (1% and 2%) to different body surface areas (from 90 to 900 cm2), to intact and abraded skin. Some of the adverse events reported were application site reactions (e.g. erythema, edema, pruritus) which were of mild to moderate intensity and appeared in both the ozenoxacin and placebo arms.

These data provide assurance for the safety of the product; specially, when the main intended indication of impetigo affects predominantly the pediatric population.

The results of the skin tissue exposure study showed that ozenoxacin appears to remain in the upper layers of skin (stratum corneum and epidermis) and does not easily penetrate to the lower skin layers (dermis). These results are consistent and complement the results of the Franz Cell studies in human skin which show a lack of percutaneous absorption of ozenoxacin from 1% ointment, 1% cream or 2% cream formulations [261, 262, 263, 264, 266], and of the phase I clinical trials conducted in healthy volunteers which show a lack of systemic absorption after topical administration of different ozenoxacin formulations (ointment and cream), different strengths (1% and 2%), different body surface areas (from 90 to 900 cm2), different number of applications (single dose and repeated dose) to intact and abraded skin [267, 268, 269, 270, 271].

The high local drug concentrations in the stratum corneum and epidermis and low or no penetration in the dermis shown in this study further support the findings of the absorption clinical trials mentioned above. Together with the microbiological profile of ozenoxacin, these chacracteristics, may represent an advantage of the product for the treatment of uncomplicated skin and skin structure infections such impetigo which affects mainly the

pediatric population. The fact that ozenoxacin concentrations were approximately two-fold greater for the twice- versus once-daily application together with the high concentrations in the upper layers of skin (stratum corneum and epidermis) and the lack of absorption, provide assurance of a safe use of a twice daily application while potentially obtaining a better therapeutic response compared with a once-daily application. These results together with the results of the *in-vitro* skin metabolism study conducted in human skin samples which found that ozenoxacin was metabolically stable and no metabolism were formed in the skin [265, 266], provide evidence of the presence of the active compound in the site of administration. This is particularly relevant for the main intended indication of impetigo which affects the upper layers of the skin [127].

As part of the development of topical formulations, it is important to evaluate dermal tolerability whether the active ingredient and/or excipients are likely to be irritants or can cause sensitization when applied to the skin. Additionally, as topical products might be applied to regions of the skin that can be exposed to sunlight, it is also necessary to determine the potential to produce phototoxic or photosensitive reactions [173]. This is particularly important for quinolones as these compounds can absorb UVA light (around 350 to 360 nm) and can cause phototoxic and photoallergic reactions [90, 91, 97, 102, 103]. The assessment of the dermal tolerability is particularly important also for the potential use of the product in pediatric patients. Although the dermal tolerability is evaluated as part of the general safety evaöluation during all the clinical trials conducted as part of the development plan, specific clinical trials should be also conducted as part of the development of a topical product to in order to evaluate the irritation, sensitizing, phototoxicity and photoallergy potential of the product.

The irritancy and sensitization potential can be, evaluated by repeat insult under application of occlusion chambers (patch test methods) over several weeks [295] and sensitization potential by means of re-challenge after an interval in a different test area. There are different variants of this technique, which allow comparing a range of formulations or concentrations of the active versus the vehicle and/or a reference where possible [181]. Phototoxicity can appear when the active product and/or the excipients of the formulation absorb light in the 240–700 nm range. This can be determined using a similar design as described for the sensitization potential; although with a smaller number of subjects and includes the addition of the application of UV light after assessment of Minimal Erythema Dosage (MED) in each subject [181]. Photoallergy (or photosensitization) can be evaluated using the method of repeat insult under occlusion with the addition of UV light and re-challenge in a new area of skin following an interval [296, 297].

The dermal tolerability studies included in this work were conducted according to recommended validated industry methods, and according to FDA Guideline recommendations for this kind of studies [298, 299].

The irritation, sensitizing potential, phototoxicity or photoallergy potential of ozenoxacin has been initially tested in preclinical studies which showed a good tolerability after topical application with no effects usually related to fluorinated quinolones (phototoxicity, photoallergy) [241, 242, 243, 244]. The dermal tolerability studies conducted in healthy volunteers showed that the ozenoxacin cream formulations tested (1% and 2%) were very well tolerated and showed no evidence of irritation, sensitizing potential, phototoxicity or photoallergy potential. These findings are in line with the safety and tolerability results found in the phase I, II, III, and I-II clinical trials conducted in healthy volunteers and adults. Although, application site effects (erythema, edema, pruritus, irritation) have been reported in some of these studies, these were of mild and moderate intensity and similar in the active and placebo groups. Moreover, the dermal tolerability studies specifically designed to evaluate the dermal tolerability of the product, have shown a very good dermal tolerability profile.

The data obtained from the absorption, safety, and tolerability data together with the data obtained from the phase II efficacy, safety, and tolerability data, allowed to select the 1 % ozenoxacin cream as the final formulation for development and marketing and was the one included in the phase III clinical trials and in the absorption, safety, and tolerability study in patients with impetigo.

Spacial considerations should also be mentioned in relation to the absorption, safety, and tolerability study conducted in adult and pediatric patients with impetigo. As commented, all the clinical studies conducted with ozenoxacin in healthy volunteers showed a lack of absorption and a good safety and tolerability profile; however, as part of the clinical development of the product, the absorption, safety, and tolerability of the product should be also evaluated in the intended indications and populations. According to regulatory requirements, the clinical development of a topical product need to include a study (or studies) to evaluate the absorption, safety, and tolerability of the final (to be marketed) formulation in the target indication applied at the intended interval of administration and intended duration of treatment. In this context, a study was designed with the objective to evaluate the absorption, safety, and tolerability of ozenoxacin 1% cream in adult and pediatric patients with impetigo [283]. The fact that the study was conducted in patients, allowed also to evaluate the initial clinical effect of the product in children and adults with impetigo.

Taking into account the very good safety and tolerability profile demonstrated in pre-clinical and clinical studies previously conducted in adults, the risk-benefit ratio for topical administration of ozenoxacin was considered acceptable in order to include pediatric patients in the clinical trials. Nevertheless, an Independent Safety Monitoring Committee (SMC) was appointed with the objective of assessing the safety of the study participants with special attention to pediatric population. The SMC was comprised by three members with expertise in clinical trials, in infectious disease, is assessment of drug safety and in

biostatistics (two experts in infectious diseases and one statistician) who were not involved in the conduct of the study and were free of any other conflicts of interest with the sponsor (financial, scientific, or regulatory). The SMC could stop the study if any signal was detected that could compromise the safety of the patients.

Also, additional measures were taken to safeguard the safety of the pediatric patients. The administration of study drug started with Group 1 (18 years to 65 years) and Group 2 (12 years to <18 years) in parallel and only progressed to Group 3 (children aged 2 to <12 years) after evaluation of the adverse events (AEs) and the laboratory tests and taking into account the advice of the SMC. Provided that no severe drug-related or unacceptable clinical or biological AEs had occurred in at least 4 patients of Group 1 and 4 patients of Group 2, the study progressed to Group 3. In the same manner, the study only progressed to Group 4 (children aged 2 months to <2 years) after evaluation of the safety data of at least 4 patients of Group 3. The decision to proceed with Group 4 was made after evaluation of the AEs and the laboratory tests and taking into account the advice of the external SMC. Provided that no severe drug-related or unacceptable clinical or biological AEs had occurred in at least 4 patients of Group 3, the study progressed to Group 4.

Also for safety reasons, the maximum area of application for the pediatric population younger than 12 years old was limited by the body surface area (BSA) (the total area did not have to exceeded a maximum of 2% of the BSA). Taking into account the effects generally related to quinolones possible in this study, apart of the general safety monitoring, special attention was paid to the musculoskeletal physical examination of patients in search of possible events on joints (arthropathy and /or tendinopathy)

The study included the evaluation of different pediatric groups as described in the ICH Guideline for the clinical investigation of medicinal products in the pediatric population [300] and was conducted taking into account the Guideline recommendations for the choice of formulations for the pediatric population [301] and for pharmacokinetics in the development of products for pediatric population [302], and the ethical considerations for clinical trials on medicinal products conducted in the pediatric population [303].

The ICH-E11 Guideline for the clinical investigation of medicinal products in the pediatric population [300] recognizes that any classification of the pediatric population into age categories is to some extent arbitrary, but recognizes that a classification is helpful for study design considerations in pediatric patients. The categorization proposed by the guideline is the following:

- Preterm newborn infants.
- Term newborn infants (0 to 27 days).
- Infants and toddlers (28 days to <24 months).
- Children (2 to <12 years).

- Adolescents (12 to <16 or <18 years (dependent on region)).

The study included patients in different age subsets taking into account this categorization, starting with patients of 2 months that could potentially benefit from a topical treatment for impetigo.

Taking into account that the main objective of the study was to evaluate the systemic bioavalability of ozenoxacin after topical applications of a topical ozenoxacin formulation, no control group (e.g. placebo group) was considered necessary. The design and characteristics of the study (including the age group distribution and number of subjects in each group) were discussed and agreed with the FDA and EMA PDCO and are part of the pediatric development plan of ozenoxacin.

The majority of plasma samples of the study (with an exception of 4 samples) were below the limit of quantitation LOQ and therefore, no pharmacokinetic parameters could be calculated. The 4 samples that showed levels above LOQ were found in 2 patients [283, 284]. These levels only slightly above the LOQ (0.5 ng/ml), seemed sporadic without following a specific pattern, and were found in 1 patient aged 2 years - 12 years and in another patient aged 2 months- 6 months. Patient 0301 was a 3 years old patient with lesions in the scalp, the right arm, and the left arm who showed an affected area of 1.17 cm^2 and had one level above LOQ at day 6 at 12 hours after last ozenoxacin administration (before the second daily administration of ozenoxacin). Patient 04201was a 3 months old patient with lesions in the face and the right arm who showed an affected area of 3.40 cm^2 and had 3 levels above LOQ at day 4 before administration of ozenoxacin, at day 4, at 1 hour after administration of ozenoxacin, and at day 6 at 1 hour after administration of ozenoxacin. Therefore, these levels could be probably explained by an accidental ingestion of ozenoxacin by these patients and an overall lack of systemic absorption could be concluded. This is in line with the results obtained in all other phase I clinical studies performed in which ozenoxacin plasma levels were below the limit of quantification in both intact and abraded skin after single and multiple administrations to different dermal extensions (up to 900 cm^2) [267, 268, 269, 270, 271].

The conduct of clinical trials in pediatric population can face different challenges such us the consenting process which should be given by parents or legal guardians, recruitment and retention of patients, compliance, and ethical issues. Some of these challenges can be is especially relevant in the case of pharmacokinetic studies with no direct benefit to the patients. This study however, was conducted in patients with impetigo in a controlled setting and the overall compliance was very good with the majority of patients exposed to the total of planned doses and achieving 100% compliance [283, 284].

Informed consent has been obtained from all participants included in the study. Prior to signing the informed consent form (ICF), the patients or their caretakers were given an opportunity to discuss any issues concerning the study with a physician who had suitable

knowledge of the study and to have all questions answered openly and honestly. The patient information sheet (PIS) and ICF was signed and dated; one copy was handed to the patient and the investigator retained a copy as part of the clinical study records. For patients legally unable to provide the informed consent (less than 18 years or minor by local law), the informed consent had to be obtained from the parent(s) or legal guardian in accordance with regional laws or regulations. Additionally, separate assent forms had to be signed and dated by minors where possible. All patients were fully informed about the study in language and terms they were able to understand (language of PIS was adapted for the appropriate understanding of the different study age groups). Specific informed consent forms were adapted for the understanding of the diferent pediatric populations included in the study. PIS and ICF were provided in the three main South African languages (English, Afrikaans, and Xhosa).

Preclinical studies conducted in extreme conditions such as daily topical administrations of high doses of ozenoxacin during 28 days to abraded skin of mini-pigs have shown negligible absorption [221]. Also, in a clinical trial conducted in healthy volunteers with applications of a high ozenoxacin cream concentration (2%) to an extended abraded skin surface (200 cm^2), no absorption has been observed [270, 271]. Therefore, no significant absorption was expected in patients with impetigo or even in young pediatric population with immature skin as the conditions of the studies mentioned above are much more extreme that the conditions found in a skin affected by impetigo or in pediatric immature skin. The findings of this study have then confirmed the results found in previous preclinical and clinical studies and provide further assurance of the safety profile of ozenoxacin.

A thinner stratum corneum and epidermis due to structural and functional differences has been described infant skin in relation to adult skin [173]. These differences would suggest a greater susceptibility of infants to percutaneous absorption of drugs. However, in the present study, no absorption was detected even in the younger children with theoretically more immature skin in relation to older children and adults.

The locations of the impetigo lesions that were treated and exposed to ozenoxacin 1% cream were the scalp, face and neck, upper and lower, lower trunk, and upper and lower extremities, were the ones expected for the clinical presentation of impetigo. Many patients presented impetigo lesions in more than one location.

Although patients with a maximum of 100 cm^2 of affected area could be included (with a limitation of not exceeding a maximum of 2% of the BSA in patients younger than 12 years), the patients included in the study presented affected areas of ranging from 1.1 to 2.4 cm^2. Although, these affected areas may be viewed as a low exposure for the evaluation of the absorption in the population affected by impetigo, they are in line with the characteristics of the lesions at baseline seen in the phase pivotal III clinical trial conducted

in adult and pediatric patients with impetigo [256, 257, 258] and in the pivotal clinical trials conducted with the retapamulin in adult and pediatric patients with impetigo which were the basis of regulatory approval of the product in this indication [304, 305, 306]. Therefore, it can be considered that the study represents the evaluation of the absorption of the product in the real clinical setting in the target population and thus representing the clinical conditions that would be candidate for topical antibiotic treatment. In addition, the product has been evaluated in much more extreme conditions such as repeated administrations of 3 times a day during 7 days, higher concentrations (2% cream), damaged skin (200 cm^2 of abraded skin abraded skin), and large extensions (up to 900 cm^2) with no absorption seen in any of these studies; therefore, no absorption would be expected in patients with impetigo even with a larger affected area than the one seen in the clinical trials (in this phase I-II clinical trial and in the phase III clinical trial).

Moreover, absorption studies conducted with other products, have shown that the surface area seem to be more important factor for systemic exposure than formulation strength [307]; therefore the available data in healthy volunteers (up to 900cm^2 in single application with the 1% ointment formulation, 100 cm^2 of three times daily applications of the 1% ointment formulation during 7 days and 200 cm^2 of twice daily application of the 2% cream to abraded skin during 7 days) is covering the maximum exposure expected for the intended indication of impetigo (up to 100 cm^2) of twice daily applications during 5 days.

Young children have limited blood volume in comparison to adults and therefore the amount of blood obtained for pharmacokinetic evaluation, and laboratory tests (hematology and biochemistry) had to be adapted to the different age groups in accordance to the limits recommended by pediatric guidelines [303]. According to these guidelines, per individual, the trial-related blood loss (including any losses in the procedure) should not exceed 3 % of the total blood volume during a period of four weeks and should not exceed 1% at any single time. The total volume of blood is estimated at 80 to 90 ml/kg body weight; 3% is 2.4-2.7 ml blood per kg body weight. Therefore, a careful evaluation of the amount of blood to be withdrawn should be taken into account in the design of a clinical trial in the pediatric population [308]. The amount of needle pricks should also be taken into amount to avoid excessive invasive and uncomfortable procedures in the children. Taking these factors into account, the amount of blood obtained for pharmacokinetic evaluation, and laboratory tests (hematology and biochemistry) had to be adapted to the different age groups in accordance to the limits recommended by pediatric guidelines [300, 303]. Based on these considerations, for pediatric patients in the younger age groups the total amount of blood obtained had to be limited while at the same time guarantying the minimum number of samples necessary to achieve the objectives of the study. The table below summarizes the blood sampling scheme for pharmacokinetic analysis for the different age groups.

Table 30. Blood sampling scheme for pharmacokinetic analysis (absorption, safety, and tolerability study in patients with impetigo)

Group	Day 1	Day 2	Day 4	Day 6	Day 7
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Group 1 18 y-65 y (n=8)	15 m pre-dose	15 m pre-dose, 1, 4, & 12 h after	15 m pre-dose, 1, 4, & 12 h after	15 m pre-dose, 0.5, 1, 2, 4, 8 & 12 h after	24 h post last dose
Group 2 12 y-<18 y (n=9)	15 m pre-dose	15 m pre-dose, 1, 4, & 12 h after	15 m pre-dose, 1, 4, & 12 h after	15 m pre-dose, 0.5, 1, 2, 4, 8 & 12 h after	24 h post last dose
2 y-<1 2 y (n=9)	15 m pre-dose	15 m pre-dose, & 1 h after	15 m pre-dose, & 1 h after	15 m pre-dose, 1, 4, & 12 h after	
Group 4 2 m-<2 y (n=6)	15 m pre-dose	15 m pre-dose, & 1 h after	15 m pre-dose, & 1 h after	15 m pre-dose, & 1 h after	

The study had a predominance of patients of black and mixed (multiracial) race (because of the geographical region in which it was conducted). All of the patients were of black race or multiracial race; no Caucasian patients were included in the study. Although no differences in the absorption of ozenoxacin was expected in skin of different racial origin, it was considered necessary to evaluate the existence of possible differences. Therefore, in order, to evaluate if the skin of black race can present differences in absorption compared to white skin (Caucasian), a percutaneous absorption study has been conducted in an *in-vitro* model of Franz cell with black and white skin obtained from different human donors [264, 266]. This in vitro model is considered adequate for predicting the in vivo percutaneous absorption of chemicals through the skin [288] and therefore allows the evaluation of potential permeability differences between different types of human skins. This study showed that the percutaneous absorption of ozenoxacin after application of 1% ozenoxacin cream was negligible in human skin from black and white skin samples. Therefore, the skin types are not expected to show differences in relation to the absorption of ozenoxacin cream and the results of the phase I-II absorption clinical trial can be considered applicable also to Caucasian population.

In fact, percutaneous absorption of drugs can be affected many factors other than skin race such as drug related factors, exposure related factors, and skin related factors. Drug-related factors include molecular weight, lipid solubility, water solubility, vehicle, cutaneous metabolism, and concomitant administration with other drugs that may serve as enhancers. Exposure related factors include drug concentration, duration, climate (temperature and humidity), and the matrix (e.g., soil). Skin-related factors include blood flow, pH, skin thickness, hair and pore density, and the content and structure of proteins, location, glycosaminoglycans (GAGs), water, and lipids [309, 310, 311, 312, 313, 314].

One published study conducted in women of different ethnic origin found that ethnicity could play a factor with elderly African American and Caucasian women (>51 years)

having increased skin dryness when compared to their Chinese or Mexican counterparts. In this case the absorption would be related to content of skin water [315]. Water molecules not bound to proteins bind to each other and are found in a tetrahedron form. If significantly more water is found in the tetrahedron form, that may result in delayed percutaneous penetration, especially for hydrophilic drugs [304, 316]. As ozenoxacin is insoluble in aqueous solutions, it could be theorized that it would be easily absorbed in dryer skins (e.g. darker skin) and therefore the phase I-II absorption study would represent a population with more potential absorption of the drug. As no absorption has been found, it is considered that the results could be extrapolated to other races such us Caucasian population.

Although differences in percutaneous absorption of drugs have been described between young children, adults, and elderly population [304, 317, 318, 319, 320, 321, 322], the present study found no differences of absorption in any of the age groups included in the study. As previous studies in more extreme conditions found no absorption of ozenoxacin, no absorption was expected even in younger children with more immature skin.

Ozenoxacin 1% cream was safe and very well tolerated in all the age groups evaluated in the study. There were no deaths, no serious AEs, no AESIs related to the joints, no topical application-site AEs or any other significant AEs during the study. Except for one case dermatitis, which was assessed as unlikely related to ozenoxacin, all adverse events recorded in the study were assessed as not related to ozenoxacin and were mild and moderate in intensity. Although sporadic clinical chemistry and hematology values above or below the reference ranges were reported, these were generally evaluated by the investigator as not clinically significant. No clinically significant changes in vital signs were reported. This is in line with the results all previous studies conducted in healthy volunteers and the good safety and tolerability profile seen in patients with SITLs [254] and in the two pivotal phase III clinical trials conducted in adult and pediatric patients with impetigo [256, 257, 258, 259, 260].

Although assessment of some signs of dermal tolerability such as erythema can be more difficult in black skin than in white, clear skin [323, 324], in the present study the patients did not show signs of dermal tolerability such us (e.g. accompanying pruritus, itching, tissue warmth) that could orient to and underlying (misdiagnosed) erythema. Additionally, a suggested alternative for the assessment of erythema in dark (pigmented skin) is to assess skin darkening (instead of erythema) by experienced investigators [324, 325] as was the case in the units that conducted the study.

Although it was not the main objective, the study included also a clinical evaluation of the patients. Overall, no patient's condition had worsened over time and no patient was withdrawn from the study because a lesion did not heal or had worsened. Approximately half of the patients (22/45) included in the study had an outcome assessed as a clinical

success which was a cure of the skin lesions. The remaining patients showed improvement (23/45 patients) of the skin lesions. No patient showed failure of the treatment. Overall a decrease in the mean skin lesion surface area was observed from Day 1 through Days 10 to 13 in all four groups. For the clinical assessment of impetigo lesions, the evaluation was made by means of the Skin Infection Rating Scale (SIRS). This scale although, not validated has been used in previous studies to evaluate the efficacy in developments of products for the treatment of impetigo and SITLs and is recognized by Regulatory Authorities as a tool for the evaluation of efficacy [326, 327]. Moreover, the SIRS was accepted by Regulatory Authorities (e.g. FDA, EMA, PDCO) as the scale to be used to evaluate the efficacy in the pivotal phase III clinical trials.

The SIRS assessment at baseline by patient is included in table below.

Table 31. SIRS at Baseline (absorption, safety, and tolerability study in patients with impetigo)

	Group 1	Group 2	Group 3	Group 4			
	18 y-65 y (n=8)	12 y-<18 y (n=9)	2 y-<1 2 y (n=9)	Overall (n=19)	12 m-<2 y (n=7)	6 m-<12 m (n=6)	2 m-<6 m (n=6)
Mean (SD)	13.3 (1.83)	14.8 (3.19)	13.7 (3.67)	13.1 (2.66)	14.9 (2.19)	13.7 (1.51)	10.5 (2.17)

The mean scores for the SIRS showed a decrease from Day 1 through Days 10 to 13 for all four groups (including the Group 4 subsets). In Group 4, the highest mean score of the skin infection rating was observed for the subset 12 months to 2 years. These results provide evidence of the pharmacological effect of ozenoxacin which has demonstrated in the two phase III clinical trials conducted in patients with impetigo [256, 257, 258, 259, 260].

The clinical studies included in the present work have been conducted as part of the clinical development plan of a topical formulation of ozenoxacin and have provided evidence of the lack of systemic absorption even in conditions which are much more extreme than the conditions for the intended indication. In fact, ozenoxacin has been evaluated in up to 900 cm^2 in normal skin and in 200 cm^2 in abraded skin; this is much higher than the maximum surface of 100 cm^2 (and a maximum of 2% of the BSA for patients <12 years) which will be treatment recommendations for ozenoxacin cream. Moreover, the abraded skin model represents a much more extreme situation than the impetigo condition that affects the outer layers of the skin.

The safety and tolerability profile of ozenoxacin is also complemented the data from the phase II and phase III clinical trials conducted in patients. In fact, ozenoxacin was found to be safe and well tolerated in the phase II study in which 199 adult patients with SITLs who received ozenoxacin cream at concentrations of 0.25%, 1% and 2% [253, 254]. Ozenoxacin was also found to be safe and well tolerated in a phase III study in adults and pediatric patients (aged 2 years and older) with impetigo in which 156 patients were exposed to ozenoxacin 1% cream [256, 257, 258] and in a second phase III clinical trial in

adults and pediatric patients (aged 2 months and older) with impetigo in which 206 patients were exposed to ozenoxacin 1% cream [259, 260].

Ozenoxacin has been evaluated in many clinical studies under different conditions:

- Different topical formulations: ointment and cream.
- Different concentrations: 0.25%, 1%, 2%.
- Different administration regimens: 1 application, 2 applications/day, 3 applications/day.
- Different treatment durations: single dose, 5 days, 7 days.
- Different skin extensions: from 9 cm^2 to 900 cm^2.
- Different skin conditions: normal, abraded skin.
- Studies in different phases (phase I, Phase I-II, phase II, Phase III).
- Different type of subjects: healthy volunteers and patients with SITLs and impetigo.
- Different populations: adult and pediatric patients distributed in different age subsets (from 2 months to < 18 years old).

In all these conditions, no absorption of ozenoxacin has been detected and a good safety and tolerability a profile has been shown. All these studies provide so far a considerable number of subjects exposed to ozenoxacin. The total number of subjects exposed to ozenoxacin to date including all studies conducted in healthy volunteers and patients is described below in the table below.

Table 32. Subjects (healthy volunteers and patients) exposed to ozenoxacin during the clinical development

Condition	N subjects
Subjects exposed to ozenoxacin (total):	**1,337**
Healthy volunteers included in absorption and dermal expositions studies	**105**
Healthy volunteers included in dermal tolerability clinical studies	**673**
Patients included in clinical trials (total)	**559**
Patients with SITLs	**151**
Patients with impetigo (total)	**408**
Adult patients with impetigo (≥ 18 years)	**119**
Pediatric patients with impetigo (2 months to <18 years)	**289**
Pediatric patients with impetigo (12 years to >18 years)	**51**
Pediatric patients with impetigo (2 months to < 12 years)	**238**

The microbiological profile of ozenoxacin, together with the preclinical data, and the results of the clinical trials conducted to date provide a favorable benefit-risk relationship for the use of ozenoxacin in adult and pediatric patients (aged 2 months and older) with impetigo.

The lack of absorption and the good safety, and tolerability results were consistent across all the clinical trials conducted with applications of different ozenoxacin formulations, different strengths to different body surface areas (from 90 to 900 cm2), to intact and abraded skin in healthy volunteers and adult and pediatric patients with impetigo.

5.8 Conclusions

5.8.1 In-vitro percutaneous absorption and metabolism

The *in-vitro* percutaneous absorption studies in human skin have shown:

- No percutaneous absorption of ozenoxacin from 1% ointment, 1% cream or 2% cream formulations.
- Negligible percutaneous absorption of ozenoxacin following application of a 1% ozenoxacin cream to human skin samples from African and Caucasian subjects indicating no influence of ethnic origin on systemic absorption.
- Metabolic stability of ozenoxacin in the skin at concentrations of 7, 35 and 70 μM.

5.8.2 Systemic bioavailability, safety and tolerability

No systemic absorption of ozenoxacin was observed in any of the four studies even with extended applications (single application of 5 g of ointment to 5% of BSA) to intact skin and repeated applications of 1 g of cream to 200 cm^2 of abraded skin). As plasma ozenoxacin concentrations were consistently below the limit of quantitation, it was not possible to derive pharmacokinetic parameters related to absorption and systemic bioavailability.

Ozenoxacin was well tolerated across all studies for both formulations (ointment and cream) and all strengths tested (1% and 2%). There were no serious adverse events or any clinically significant abnormal findings in laboratory values, ECG results or vital signs in any study. The intensity of all reported adverse events with a probable or possible relationship to study medication was mild to moderate.

5.8.3 Skin tissue exposure

Ozenoxacin formulated as a 2% cream and applied once or twice daily appears to remain in the upper layers of skin (stratum corneum and epidermis) and does not easily penetrate to the lower skin layers (dermis). Concentrations in the skin were higher with twice daily than with once daily applications.

Repeated topical applications of ozenoxacin were well tolerated. No serious adverse events were observed and there were no clinically significant abnormal physical examination, vital sign, ECG or clinical laboratory results recorded during the study. All adverse events reported during the study were considered to be unrelated to study medication.

5.8.4 Dermal tolerability studies

Ozenoxacin 1 and 2% creams showed excellent dermal tolerability and safety profiles when applied topically under maximum occlusive patch conditions, with little or no evidence of irritation, sensitizing potential, phototoxicity or photoallergy.

5.8.5 SYSTEMIC BIOAVAILABILITY AND SAFETY IN IMPETIGO

No significant plasma levels were observed after applications ozenoxacin 1% cream to patients with impetigo in any of the different age groups included in the study (2 months to 65 years old).

Ozenoxacin 1% cream was safe and well tolerated in all age groups included in the study.

All the patients showed clinical cure or improvement of the lesions; no patient showed a worsening of the lesions.

The lack of systemic absorption and good safety and tolerability profile observed in this study conducted in patients of different age groups with impetigo are in line with previous clinical studies conducted in healthy volunteers and with *in-vitro* permeation studies conducted with human skin samples.

5.8.6 GENERAL CONCLUSIONS

The following conclusions are derived from the different studies conducted as part of clinical development of ozenoxacin and included in the present work:

- No absorption of ozenoxacin has been detected and a good safety and tolerability profile has been observed after application of different topical formulations (ointment and cream), concentrations (1%, 2%), administration regimens (1 single application, 2 applications/day, 3 applications/day), treatment durations (1 day, 3, days, 5 days, 7 days) applied to different skin extensions (from 9 cm^2 to 900 cm^2), and skin conditions (normal, abraded skin).
- Ozenoxacin cream does not easily penetrate to the lower layers of skin (dermis) and appears to remain in the upper layers of skin (stratum corneum and epidermis).
- Ozenoxacin cream formulations show no irritation, sensitization, phototoxic and photoallergic potential.
- Ozenoxacin 1% cream shows a lack of absorption and a very good safety and tolerability profile when applied to adults and pediatric patients affected by impetigo.
- The microbiological profile of ozenoxacin, together with the preclinical data, and the results of the clinical trials, confirming the lack of absorption and the good safety and tolerability profile in the target population, provide a favorable benefit-risk relationship for the use of ozenoxacin 1% cream in adult and pediatric patients (aged 2 months and older) with impetigo.

All these data together with the efficacy results from the Phase II and the two phase III clinical trials should serve as the basis of regulatory applications for marketing approval for the indication of impetigo in adult and pediatric patients (aged 2 months and older).

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