Preclinical Pharmacokinetics and Efficacy of Debio 1450 (Previously AFN-1720), a Prodrug of the Staphylococcocal-specific Antibiotic Debio 1452 (Previously AFN-1252)

N. Kaplan[1,3] and B. Hafkin[2,3]

Affinium Pharmaceuticals, [1]Toronto, ON, Canada and [2]Austin, TX, USA, Debiopharm International SA[3], Lausanne, Switzerland

gregoire.vuagniaux@debiopharm.com

nachum.kaplan@consultant.debiopharm.com

Introduction and Purpose

Debio 1450 (previously known as AFN-1720) is a recently developed prodrug of Debio 1452 (previously know as AFN-1252) which targets FabI in the staphylococcal fatty acid biosynthesis cycle, an essential target in this organism. Debio 1452 has highly potent specific-spectrum activity against staphylococci, including all know drug-resistant mechanisms e.g. MRSA and VISA strains, with an MIC_{90} of 0.016 µg/ml against >5000 strains. Debio 1452 has also excellent efficacy in mouse models of infection including thigh abscess, skin abscess, granuloma pouch, pneumonia and septicemia models. AUC/MIC was previously determined to be the PK/PD driver for Debio 1452.

Debio 1452 has recently completed an oral Phase 2a study in Acute Bacterial Skin and Skin Structure Infections (ABSSSI) due to *Staphylococcus* with overall cure rates of 93% and an excellent safety profile. Debio 1450 has recently completed a Phase 1 study using a sterile IV solution (see poster P1716).

Debio 1452 is a BCS Class II drug with limited aqueous solubility and oral bioavailability, whereas its prodrug Debio 1450 has a dramatically improved oral bioavailability and solubility profile. This study examines the Debio 1450 *in vitro* antibacterial activity, preclinical pharmacokinetics (PK) including conversion rates to the active Debio 1452, and efficacy in a neutropenic mouse thigh infection model.

Methods

Microdilution MICs were performed according to CLSI guidelines.

Debio 1450 was formulated in 5% dextrose in water for all *in vivo* studies. Debio 1452 and linezolid were used as comparators in the *in vitro* and *in vivo* studies; vancomycin and ciprofloxacin were tested in the *in vitro* studies as well.

PK studies in Sprague-Dawley Rats and Beagle Dogs were performed after both IV and oral administration, and plasma samples collected at designated time points were analyzed simultaneously for both Debio 1450 and Debio 1452 using a GLP-validated HPLC/MS/MS method. PK parameters were estimated with WinNonLin v. 6.3 using a non-compartmental approach.

For the mouse thigh model, mice were rendered neutropenic by use of cyclophosphamide. The challenge organism was *S. aureus* ATCC 29213. Mice received $5.9x10^5$ CFU/thigh via intramuscular injection in the right posterior thigh muscle 2 hours prior to dosing or the first tissue harvest (-2 hr). Debio 1450 was dosed both IV and orally at dose levels of 0.3 - 100 mg/kg. At 24 hr post-dose, mice were harvested for thigh tissue and samples were diluted and plated to provide colony counts. Efficacy was calculated as the difference of the CFU/thigh at 24 hr post-dose *vs.* the time 0 control. Predicted efficacy was modeled using the WinNonLin Inhibitory Effect Sigmoid model. PK studies were conducted in infected mice as described above.

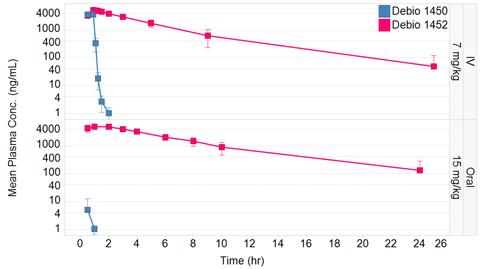
Results

In Vitro Activity of Debio 1450

Organism	Strain / Phenotype	MIC (µg/ml)				
		Debio 1450	Debio 1452	Vancomycin	Linezolid	Ciprofloxacin
S. aureus	3104; MSSA	>16	0.015	0.5	2	0.25
	3265; HA-MRSA	16	0.004	1	2	>64
	2294; CA-MRSA	16	0.03	1	2	>64
E. faecalis	101; VSE	>16	>2	1	1	0.5
	4158; VRE	>16	>2	>64	1	64
S. pneumoniae	1195; PSSP	>16	>2	0.12	1	0.5
S. pyogenes	6179	>16	>2	0.25	1	0.5
E. coli	102	>16	>2	>64	64	0.008

- **Debio 1450, as expected, showed little to no activity against all species**
- **Debio 1452 showed the expected specific-spectrum activity against *S. aureus***

Time-concentration Profiles of Debio 1450 and Debio 1452 Following Administration of Debio 1450 in Beagle Dogs



- **Debio 1450 was rapidly converted to Debio 1452**
- **Similar results in rat and mouse including higher dose levels (up to 160 mg/kg)**

Acknowledgments

We are grateful for the excellent contributions of Michael Dority and the team at In Vivo, Michigan State University to the mouse neutropenic thigh model model, Charles River Laboratories, Montreal, QC to the PK studies and Micromyx, Kalamazo, MI to the *in vitro* MIC studies

Results

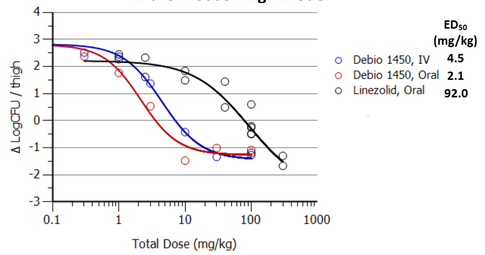
Mean PK Parameters of Debio 1450 and Debio 1452 following Administration of Debio 1450

Species	Route*	Debio 1450 Dose (mg/kg)	Debio 1450 (prodrug)						Debio 1452 (active metabolite)				
			Tmax (hr)	Cmax (ng/ml)	Half life (hr)	AUClast (hr*ng/ml)	Vz (ml/mg)	Cl (ml/hr/mg)	Tmax (hr)	Cmax (ng/ml)	Half life (hr)	AUClast (hr*ng/ml)	Relative Oral Bioavailability
Rat	IV	7	0.5	4,233	0.12	2,659	440	2,632	1.1	5,515	1.1	12,117	
	Oral	7	Not evaluable, all plasma concentrations <LLOQ						1.0	2,486	2.0	14,223	117%
Dog	IV	7	0.7	3,899	0.10	2,755	374	2,610	1.0	5,176	3.4	25,503	
	Oral	15	0.7	3.6	Not evaluable				1.5	5,178	4.4	32,378	59%

* IV administration by 1 hour infusion; oral administration by gavage

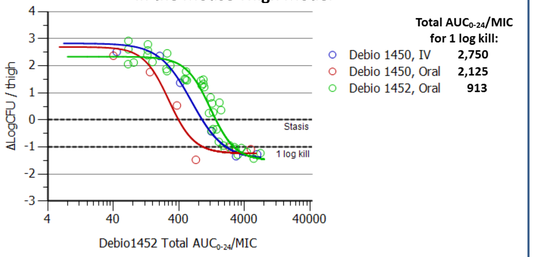
- **Debio 1450 was rapidly converted to Debio 1452 with short half life and high clearance values**
- **The prodrug delivered Debio 1452 effectively with appropriate exposures, half lives and good oral bioavailability**
- **Debio 1452 exposures were linear with dose in all species and routes of administration (tested up to 160 mg/kg, data not shown)**

Efficacy of Debio 1450 and Linezolid against *S. aureus* in the Mouse Thigh Model



- **Debio 1452 after conversion from Debio 1450 was:**
  - **Highly efficacious in the mouse thigh model**
  - **More potent than linezolid on a mg/kg basis**

PK/PD of Debio 1450 and Debio 1452 against *S. aureus* in the Mouse Thigh Model



- **Debio 1452 after conversion from Debio 1450 showed:**
  - **Excellent PK/PD index values**
  - **An improved PK/PD index value compared to Debio 1452 dosed by itself orally**

Conclusions

- **Debio 1450 was rapidly converted *in vivo* to the active metabolite Debio 1452**
- **Debio 1452 showed linear PK and excellent oral bioavailability and half-lives**
- **The *in vivo* antibacterial activity of Debio 1450, which was quickly converted to its active form, was better than Debio 1452 administered by itself orally**
- **These data highly support the continued development of Debio 1450 as a therapeutic for serious staphylococcal infections**