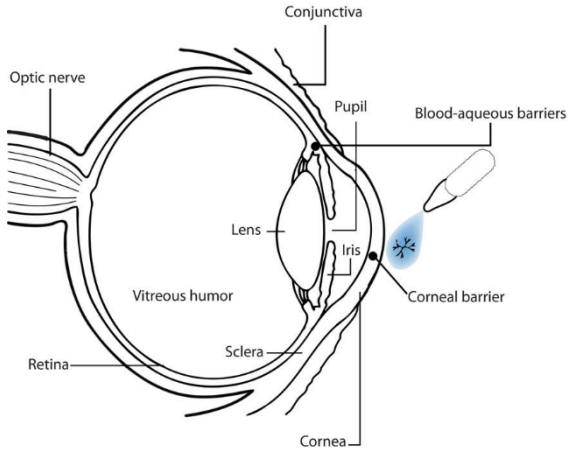
Non-Clinical Tools For In Vitro-In Vivo Correlation (IVIVC)

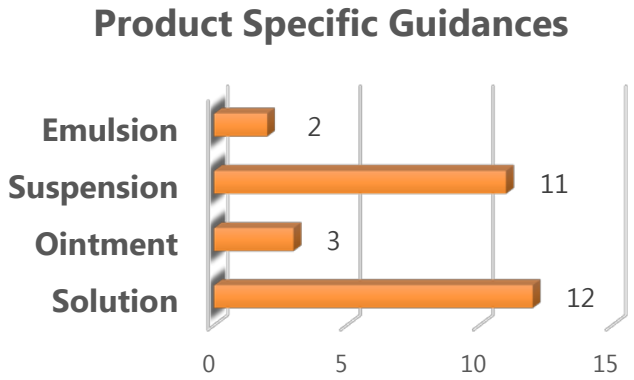
-Supporting Bioequivalence (BE) of Ophthalmic Products

Vatsala Naageshwaran
V.P. Corporate Development

BE Challenges for Product Development



CHALLENGES!



Aqueous Humor PK Studies

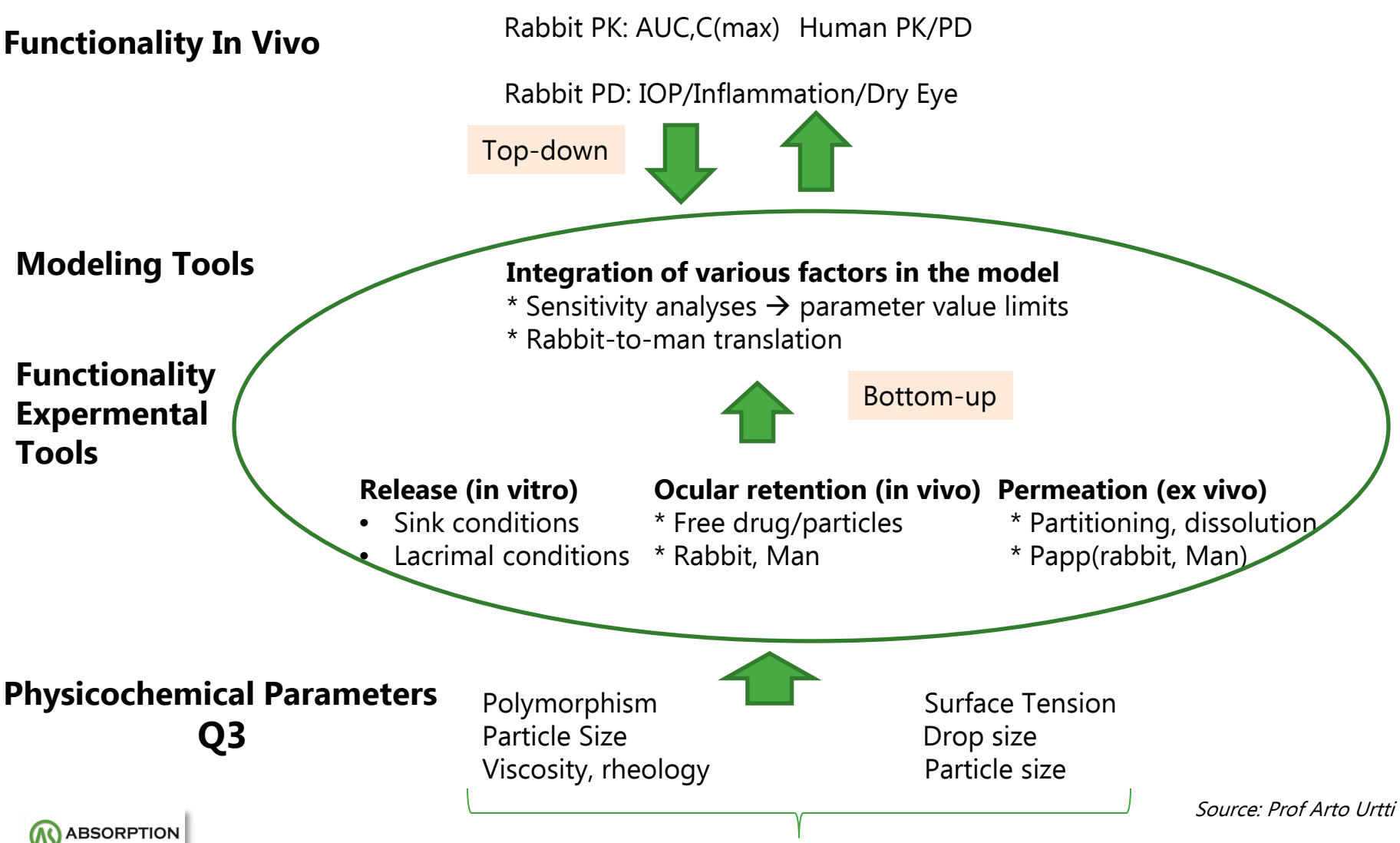
Clinical endpoint BE studies

- Uncertainty of Testing Methodologies
- Lacks correlation to In Vivo Performance
- Not on critical path for RLD approval

Q3 Characterization

Matching physicochemical characteristics to ensure equivalence in (in vivo) performance

Integrating Bio-Assays to establish IVIVC



Source: Prof Arto Urtti

Scientific Evidence for RLD Approval

IVPT
Multifactorial output

- Net Flux
- Donor Free drug concentration with time course profile
- Association with tissue (cornea and conjunctiva)
- Selective retention in tissue
- Partitioning (% permeated/% associated)

Non-clinical PK
Major and Minor compartments

- Tears, Aqueous Humor, Cornea, Conjunctiva, Iris Ciliary Body
- Adnexa, lids, lens, lacrimal glands, trabecular meshwork
- Bioanalysis – measured concentrations
- PK parameters (Cmax, AUC)

Non-clinical PD
Correlates with human efficacy

- IOP
- Inflammation
- Corneal Dryness
- Tear volume and composition
- Bacterial/viral counts
- Cytokines
- Molecular Markers

Clinical data supports model translatability

NDA # 204251 Reviewer: McDougal, Andrew

Study title: **Ocular tissue distribution study of brinzolamide (AL-4862) and brimonidine (AL-8923) following topical ocular administration of brinzolamide 1% / brimonidine 0.2%, AZOPT, or brimonidine (Falcon) to New Zealand White/Red F1 cross rabbits**

Study no.:	TDOC-0014507
Study report location:	NDA module 4.2.2.3 Pharmacokinetics – Distribution
Conducting laboratory and location:	Alcon Research, Ltd. Forth Worth, Texas 76134
Report date:	February 20, 2012
GLP compliance:	No
Drug, lot #:	• Brinzolamide 1% / Brimonidine 0.2%, lot # 18543-01 (purity not reported) • Falcon's brimonidine 0.2%, lot # 18300F • Azopt, lot # 171289F

NDA # 204251 Reviewer: McDougal, Andrew

Study title: **Effects of brinzolamide on aqueous humor dynamics in pigmented rabbits (contract study)**

Study no.:	002:41:0301
Study report location:	NDA module 4.2.1.1 Primary pharmacodynamics
Conducting laboratory and location:	[redacted]
Report date:	Signed April 17, 2001
GLP compliance:	No
Drug, lot #:	1% brinzolamide (purity and formulation not reported)

NDA # 204251 Reviewer: McDougal, Andrew

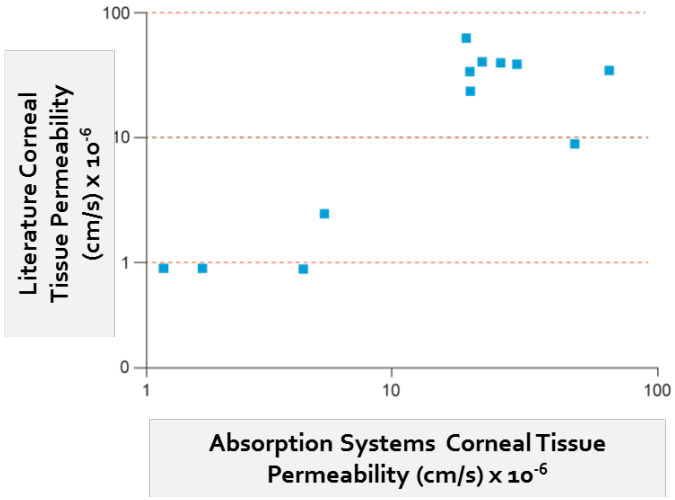
This formulation is identical to the clinical formulation

Species/Strain:	Rabbit – New Zealand White x Red, F1 *Oryctolagus cuniculus*
Number/Sex/Group:	4/sex/group
Age:	4 to 5 months at initiation of dosing
Weight:	3.0 to 3.3 kg at initiation of dosing

In Vitro Permeability/Flux (IVPT)

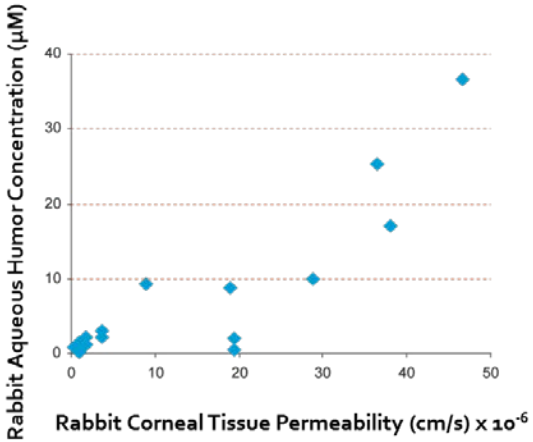
Ex Vivo Permeability Overview

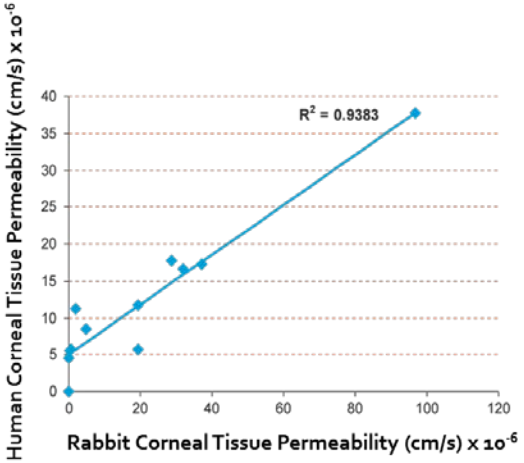
- Dutch-belted pigmented or New Zealand White rabbits
- After euthanasia, corneal and/or conjunctiva tissue is harvested



Characterization and Validation

- Morphology
- Esterase expression
- Transporter expression
- Permeability of model compounds
- Effect of strain
- Rabbit versus human

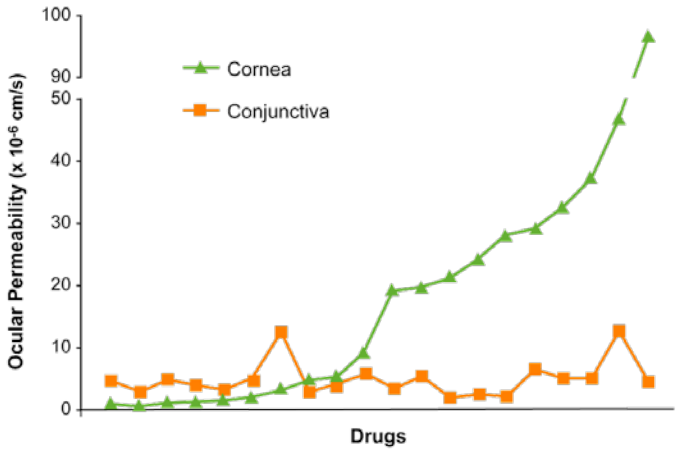




IVPT Validation– Sensitive, Selective and Reproducible

	Cornea P_{app} ($\times 10^{-6}$ cm/s)	SD	Conjunctiva P_{app} ($\times 10^{-6}$ cm/s)	SD
Acebutalol	4.68	0.39	3.24	0.94
Acetazolamide	1.28	0.26	3.39	1.23
Apraclonidine	3.11	1.78	12.6	4.74
Atenolol	1.84	0.46	4.95	1.19
Betaxolol	32.0	4.42	5.24	1.94
Brimonidine	28.8	1.22	6.73	2.03
Brinzolamide	0.91	0.93	5.15	1.28
Bufarolol	19.0	4.56	3.58	0.58
Ciprofloxacin	0.42	0.35	4.48	3.31
Clonidine	46.7	8.73	12.6	4.52
Dexamethasone	5.08	0.71	4.38	0.22
Dexamethasone Acetate	BLQ	N/A	BLQ	N/A

	Atenolol		Antipyrine	
	DB Cornea %CV	DB Conjunctiva %CV	DB Cornea %CV	DB Conjunctiva %CV
Week 1	28%	25%	16%	40%
Week 2	25%	19%	6%	18%
Week 3	32%	22%	8%	48%
Week 4	40%	45%	12%	53%
Week 5	38%	33%	9%	27%
Week 6	27%	47%	6%	24%
Week 7	19%	27%	9%	10%
Week 8	26%	26%	7%	9%
Week 9	59%	32%	47%	39%
Week 10	24%	38%	6%	20%
Average	32%	31%	13%	29%
Range	19-59%	19-47%	6-47%	9-53%
Median	28%	30%	8%	26%



Corneal range: 0.42-97, 230-fold

Conjunctival range: 1.9-12.6, 6.6-fold

ABSORPTION SYSTEMS®

IVPT - In Vitro In Vivo Correlation

Test Compound	Human Cornea		Rabbit Cornea		Human Corneal Orbs	
	Mean*	SD*	Mean*	SD*	Mean*	SD*
Latanoprost	<0.01	0.0	0.07	0.14	34.4	11.9
Latanoprost acid	37.7	14.3	96.8	83.0	9.06	3.23
Acebutolol	8.43	3.96	3.62	0.51	1.33	0.63
Brimonidine	17.7	2.7	28.8	1.22	15.8	1.2
Ciprofloxacin	5.53	1.91	0.42	0.35	Not tested	
Timolol	17.3	1.31	18.9	3.23	17.0	2.7
Lucifer Yellow	5.61	1.84	0.51	0.3	3.05	0.81
Atenolol	11.2	3.03	1.84	0.46	4.63	2.75
Antipyrine	25.7	1.61	35.1	7.14	29.3	15.9

Human cornea and Rabbit cornea show similar esterase activity

Treatment Group	**Test 1 ([redacted]) BAK free**					
Replicate	1	2	3	4	**Mean**	SD
Flux (ng/cm^2/min)	7.87	24.5	0.81	1.19	**8.58**	11.1
PE Atenolol P_{app} (10^{-6} cm/s)	0.0326	0.983	0.615	0.320	**0.488**	0.407
Treatment Group	**Test 2 ([redacted]) BAK free**					
Replicate	1	2	3	4	**Mean**	SD
Flux (ng/cm^2/min)	NC	0.258	0.205	0.261	**0.241**	0.0317
PE Atenolol P_{app} (10^{-6} cm/s)	0.173	0.434	0.0913	0.129	**0.207**	0.155
Treatment Group	**Lumigan®**					
Replicate	1	2	3	4	**Mean**	SD
Flux (ng/cm^2/min)	21.7	3.96	3.84	24.6	**13.5**	11.2
PE Atenolol P_{app} (10^{-6} cm/s)*	3.10	1.67	1.06	4.04	**2.47**	1.35

NC = not calculated due to poor linearity of the flux profile (R^2 <0.9).

* PE atenolol for all replicates dosed with Lumigan technically failed the corneal criterion, with P_{app} higher than the cut-off of 1.0×10^{-6} cm/s.

Formulation:
Lumigan® (bimatoprost 0.01%) has 4-fold higher amount of benzalkonium chloride (BAK) (0.02%). BAK is known to increase the transcorneal drug penetration by altering TJN in the corneal epthelium

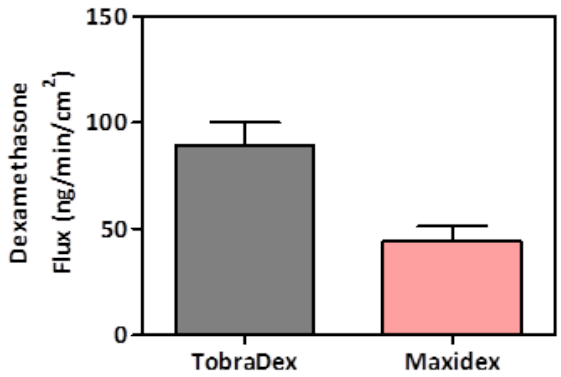
Clinical Effect:
In a 12 -month clinical study with bimatoprost ophthalmic solutions 0.01%, the most common adverse reaction was conjunctival hyperemia (31%).

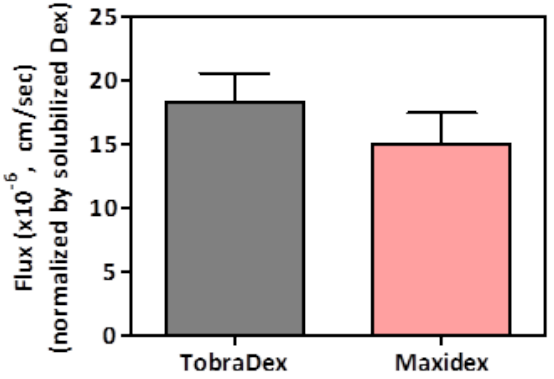
Lumigan [package insert]. Irvine, CA: Allergan, Inc. 2006

IVPT – Sensitive and Discriminatory

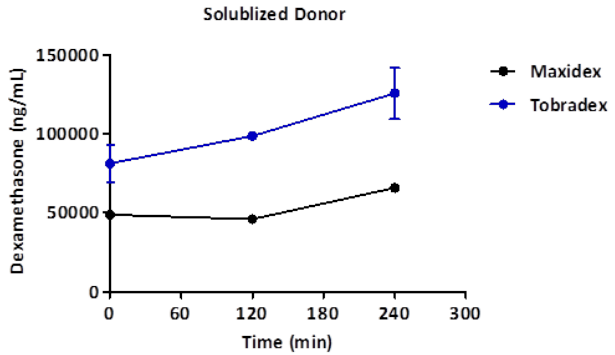
Free donor concentration

Tobradex® vs Maxidex®



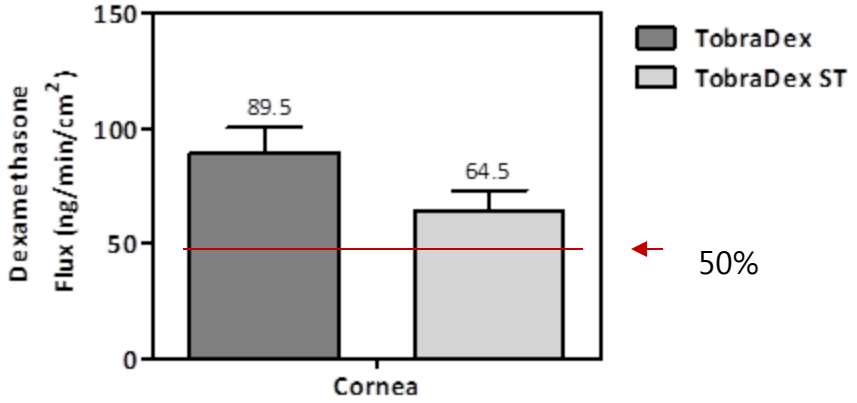


Product	Tobradex	Maxidex
Dose	0.1% (dex)	0.1% (dex)
Posology	One drop instilled into the conjunctival sac(s) every 4 to 6 hours while the patient is awake	Maximum daily dose (2x 30µl drops x 4 times per day = about 0.240 mg/day dexamethasone)

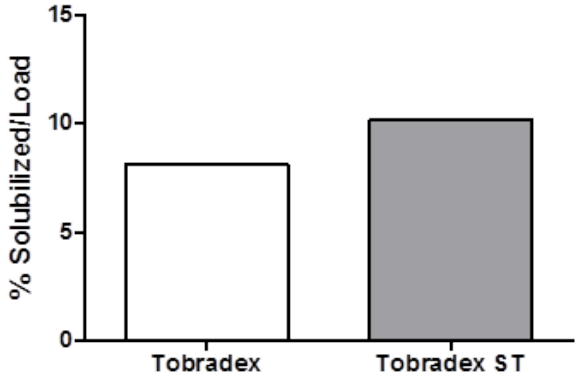


Donor (ng/mL)	Maxidex	Tobradex
0 min	48800	81200
120 min	45900	98500
240 min	65800	125500

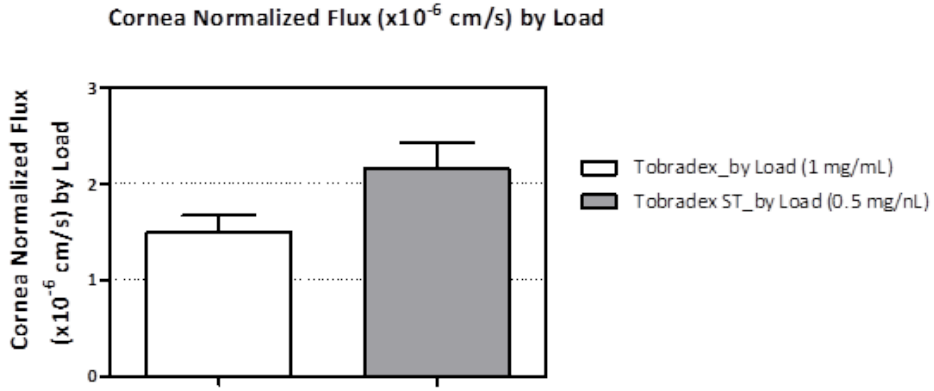
IVPT – Impact of Formulation Differences



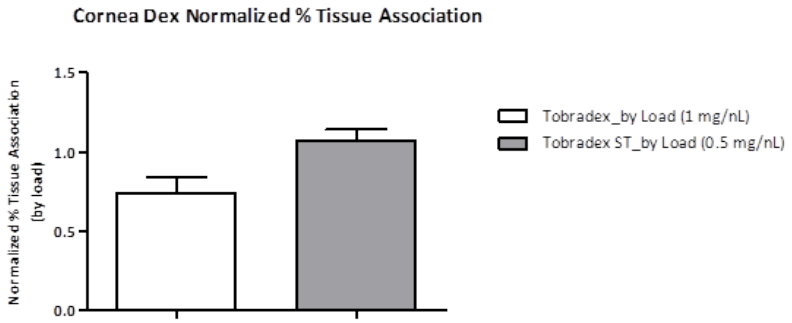
ST disproportionately higher



Free drug solubilized to begin with

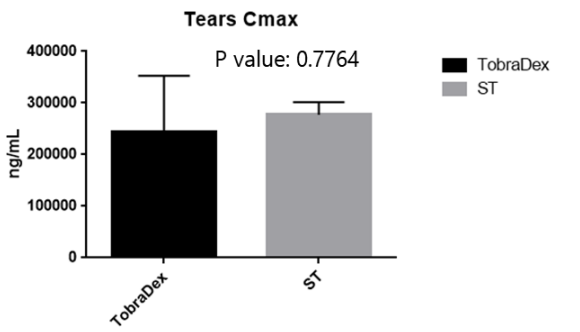


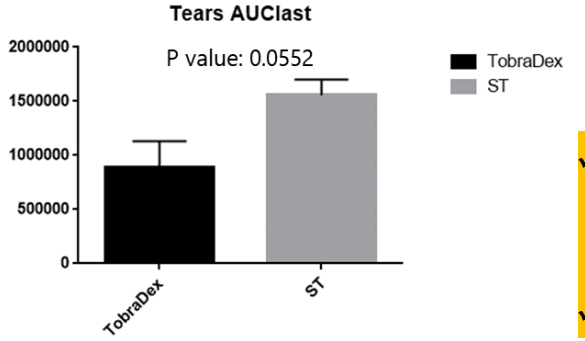
Flux normalized to load



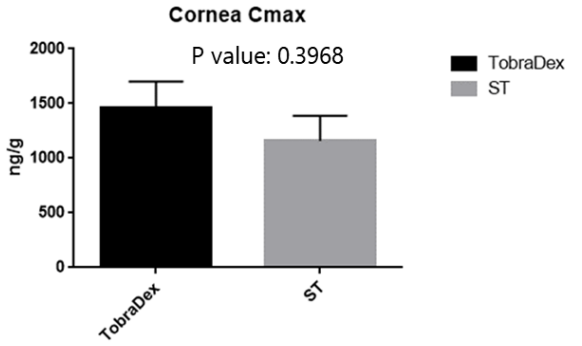
Association with cornea is more – driving factor

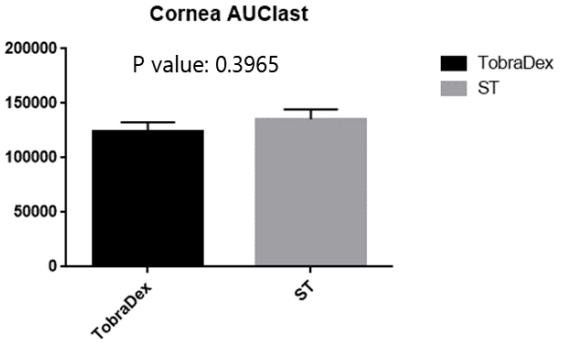
IVPT Correlates with PK- Cmax and AUC



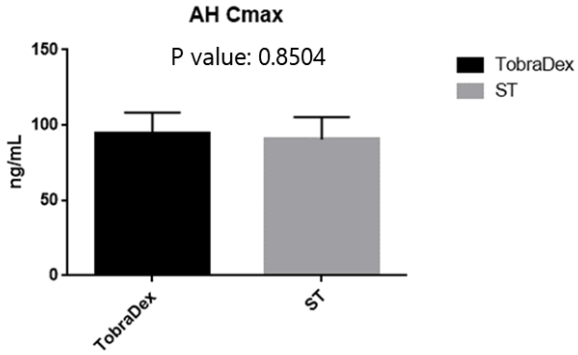


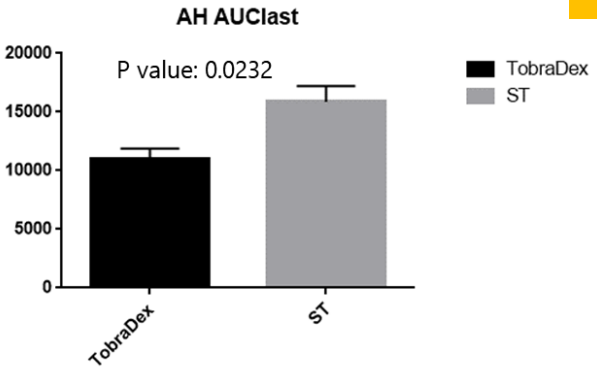
- ✓ Rabbit data shows no significant difference in Cmax between Tobradex and Tobradex ST
- ✓ Matches Human Data





- ✓ Rabbit PK data shows no significant difference in AUC between Tobradex and Tobradex ST
- ✓ Matches Human Data





Bio-Assays are Critical for Confirmation of Equivalence

- Link API and Formulation to their biological effect
- Evaluate the combined impact of discrete physicochemical characteristics
- Interplay of pre-corneal dynamics, multiple target tissues and complex differential rate processes that adjust continually to equilibrium
- Provide scientific evidence that is congruent with requirements for RLD approval
- Supports expected equivalence in human efficacy providing confidence to regulators, clinicians and patients