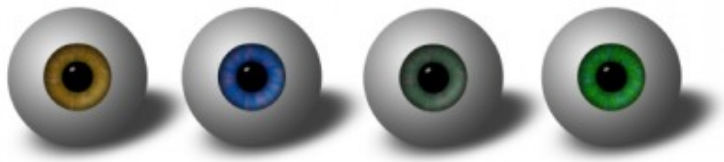
Ocular melanin modulates pharmacokinetics and drug disposition of therapeutic agents

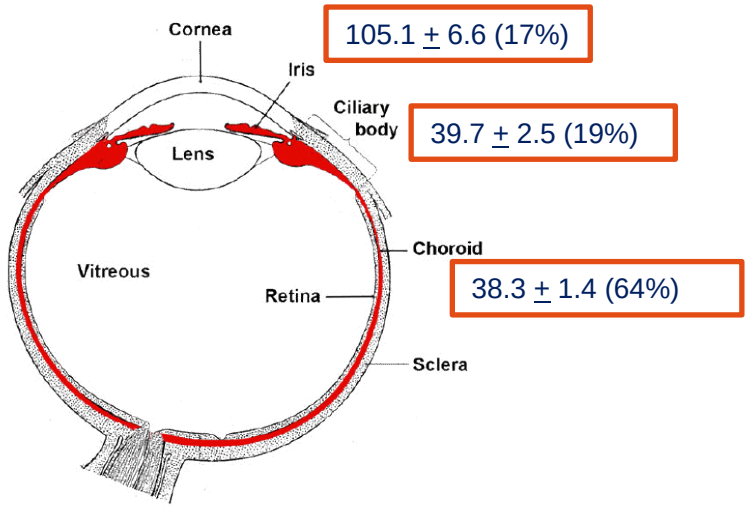
Viral Kansara, Ph.D.
Novartis Institutes for Biomedical Research, Inc.
Ophthalmology 2014

NOVARTIS

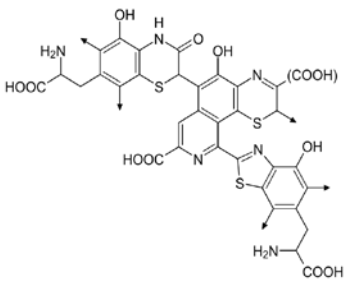


Biopolymer melanin is in front and back of the eye

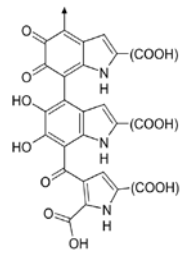
- Melanin is a **heterogenous bio-polymer** of Pyrrol containing free carboxyl and phenolic hydroxyl groups
- Synthesized from **Tyrosine** and **Cysteine** via enzymatic and polymerization steps
- Melanin acts as a free radical scavenger and photo-oxidation protector; Protects from **UV light-induced damage**
- Ocualr melanin exists in two forms: **pheomelanin (red)** and **eumelanin (black)**
  - Uveal tract – Pheomelanin > Eumelanin
  - RPE – Eumelanin > Pheomelanin
- In vitro studies suggest that the greater the **eumelanin** to pheomelanin ratio, the more **anti-oxidative** and less photo reactive the pigments
- Some evidence suggests that light-colored eyes are at higher risk for the occurrence of uveal melanoma and AMD[1]



Melanin content in human brown eyes
ug/mg of tissue; mean ± SEM (% of total uveal melanin)
J Ocular Pharm; PMID: 1402293



Pheomelanin



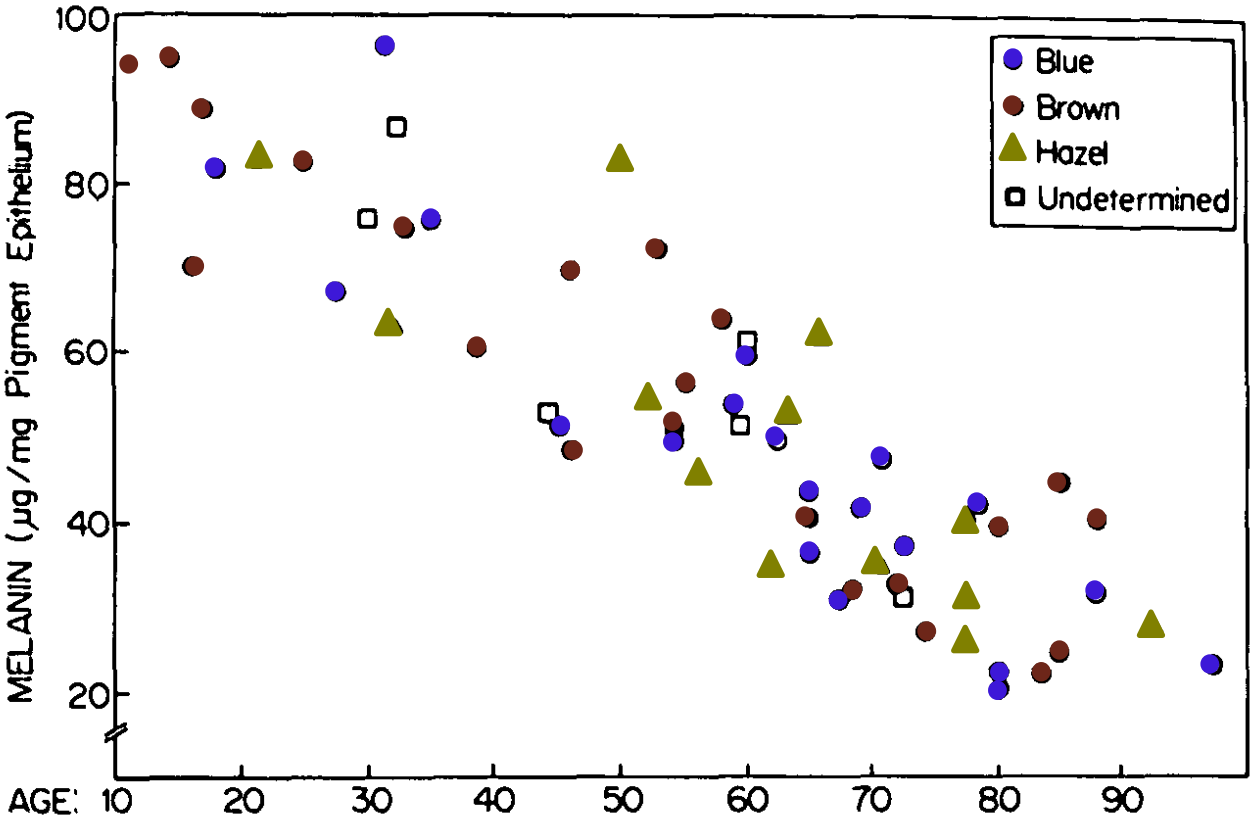
Eumelanin

[1]*Age-Related Eye Disease Study Research Group, 2000; Frank, 2000; Friedman, 1999; Klein, 1995, 2003, 2006; Sandberg, 1994; Weiter, 1985*

NOVARTIS

Melanin levels in human RPE decrease with age

Melanin concentration versus age of donors



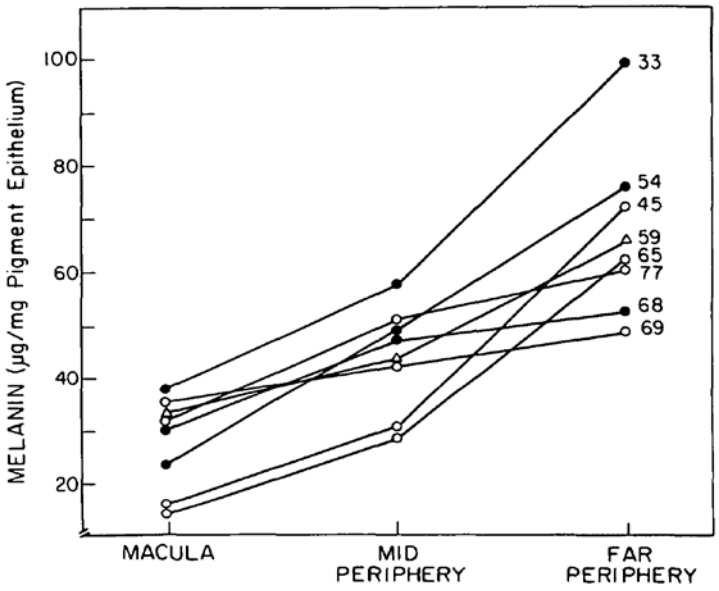
- Each value represents data from a single donor; in 12 cases, where the two eyes of a given donor were analyzed separately; a single data point represents the mean of the two values.

➢ Reduction could be due to biochemical degradation and melano-lipofuscin complex formation

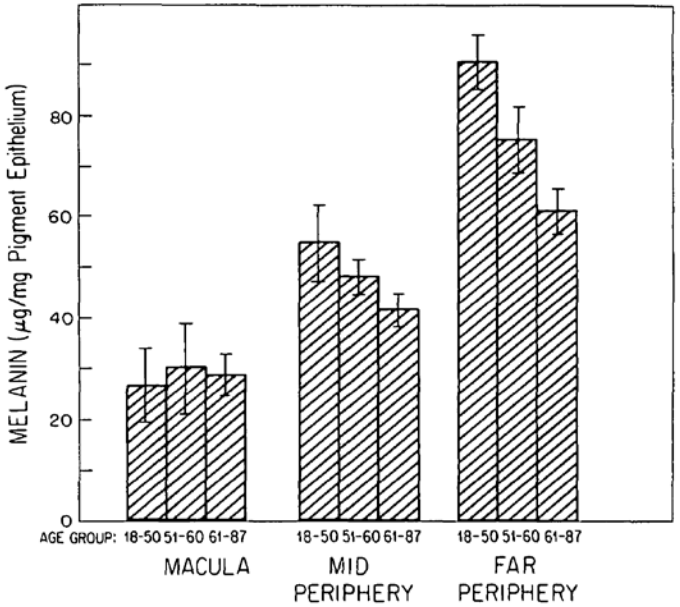
Schmidt and Peisch. Invest Ophthalmol Vis Sci 1986; 27:1063-1067

NOVARTIS

Melanin level is lower in macula than in periphery of normal human RPE



Melanin concentrations in three different regions of pigment epithelium in **eight postmortem human eyes** from donors 33-77 years of age.



- N = **16 donor eyes** grouped according to age (Five eyes from donors 18-50 years of age; four eyes from donors 51-60 years of age, and seven eyes from donors 61-87 years of age)
- Bars represent the mean ± S.E.M.

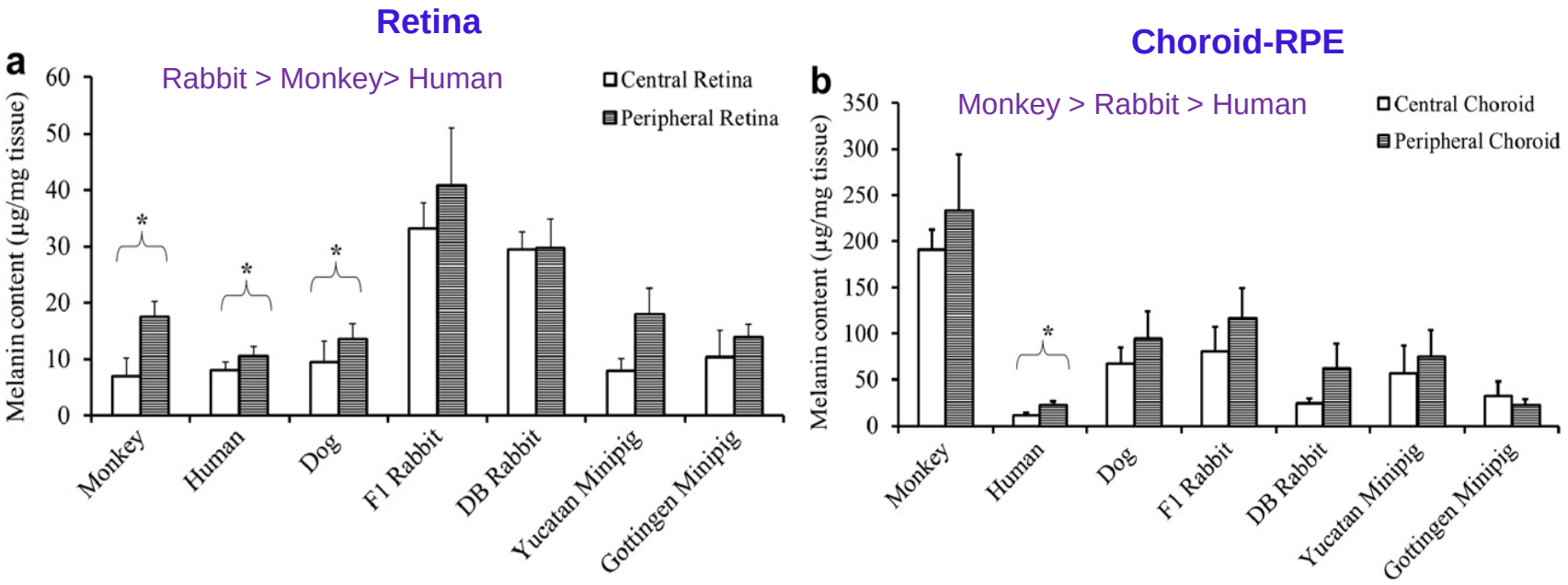
*RPE cells were harvested from post-mortem eyes

Schmidt and Peisch. Invest Ophthalmol Vis Sci 1986; 27:1063-1067

NOVARTIS

Melanin content varies among different preclinical species and strains

Regional differences in the melanin pigment content of (a) retina and (b) choroid-RPE of human



Study limitations: Small sample size (n= 3 to 6 eyes)

Overall trend for melanin content in retina + choroid: Monkey > Rabbit > Human

Kompella et al. Experimental Eye Research 2012; 98(1):23-27

NOVARTIS

Ocular melanin binding impacts drug disposition in the eye

➢ **Ocular PK**

- Melanin in iris/ ciliary body may impact anterior segment exposure,
  e.g. Antiglaucomatous: Timolol topical drop

- Melanin in RPE-choroid impacts posterior segment exposure e.g. NVS-1 rat PK (BN/SD AUC fold difference: 7x (PEC), 59x (Retina), 2x (Plasma)

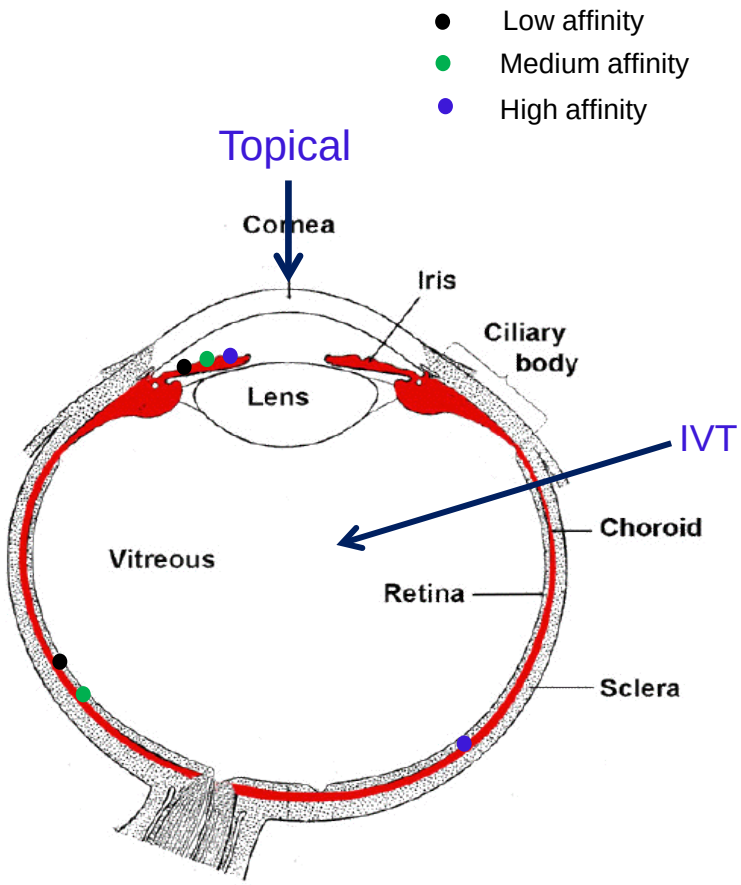
➢ **Efficacy / PD**

- Free drug (F_u) available at the site of action

➢ **Safety**

- Local drug accumulation

➢ Understanding of melanin binding characteristics may also help:
  - Explain PK/PD disconnect
  - Modeling & Simulation



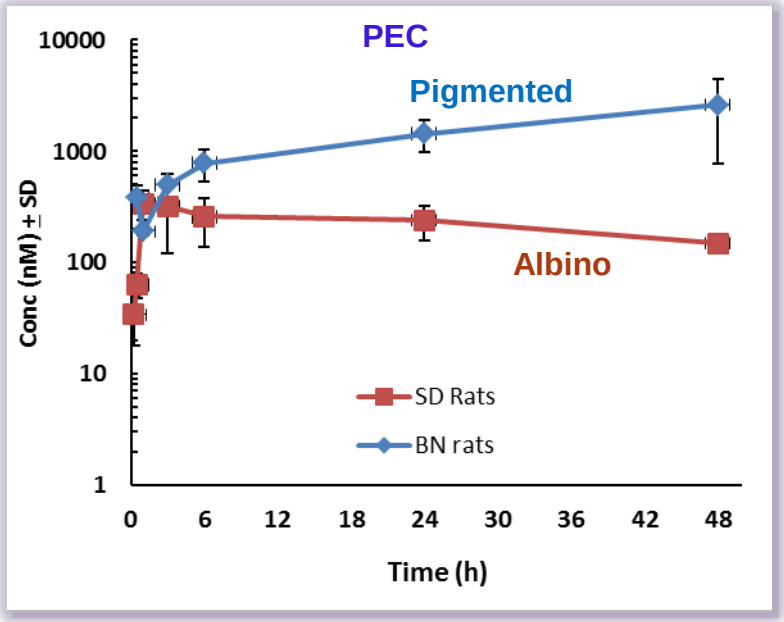
Hypothetical scenario of drug disposition following topical and IVT administration

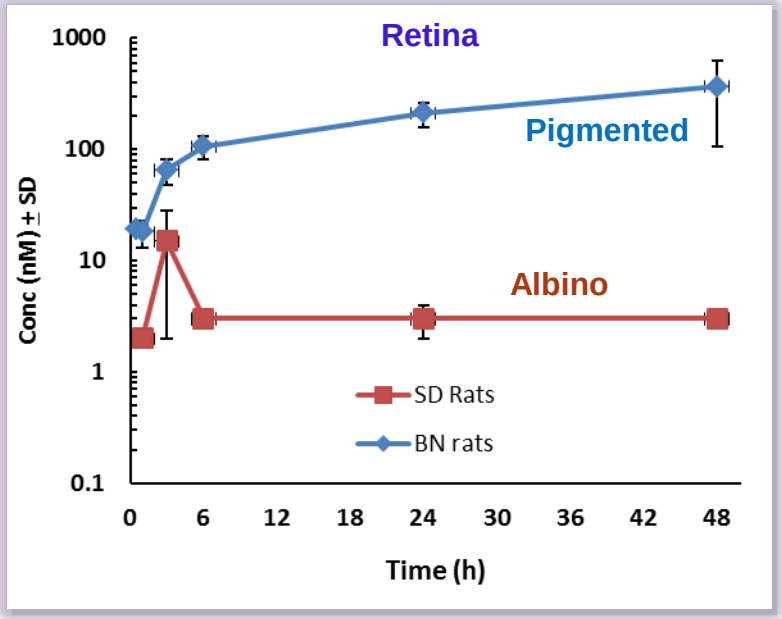
NOVARTIS

Case study: Impact of melanin binding on ocular PK

NVS-1 exhibited different ocular PK profiles in pigmented and albino rats upon PO dosing

- Brown Norway and Sprague-Dawley Male rats Male rats; N=2 rats or 4 eyes /time point
- PO dosing; 10 mpk; Formulation: 0.5% CMC/ 0.5% Tween80
- Samples: Retina, Posterior Eye Cup (RPE/choroid, sclera), Plasma





Ratio(s) BN/ SD	AUC Ratio	Cmax Ratio
Plasma	2.0	0.9
PEC	6.6	7.7
Retina	59.3	24.4

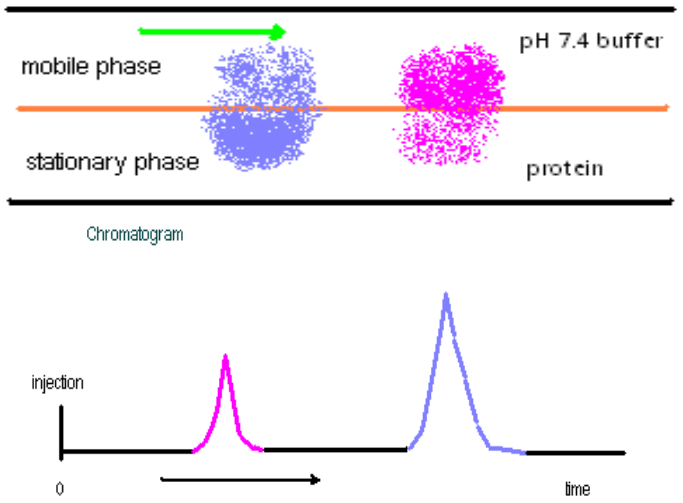
❖ **Melanin binding may be responsible for >3 fold higher AUC and longer retention in ocular tissues of BN rats**

NOVARTIS

Melanin affinity based chromatography *in-vitro* methodology

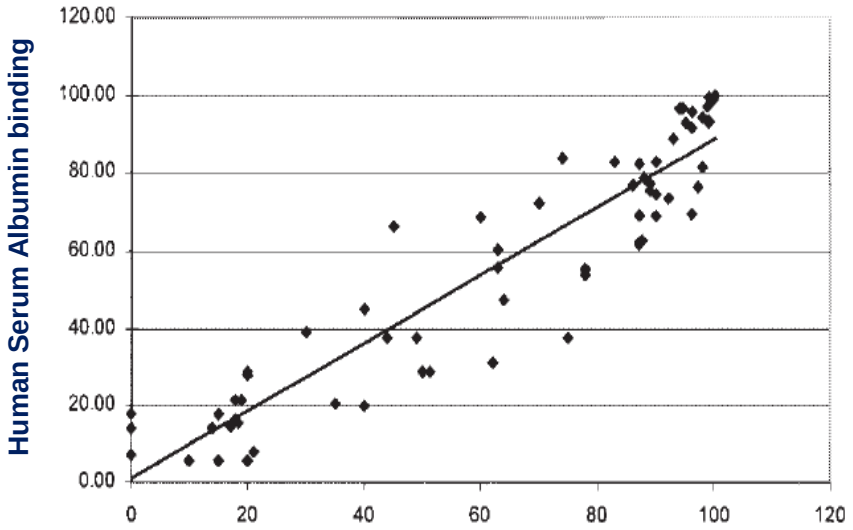
Basis of Affinity Trend analysis

- Commercially available columns e.g Human Serum Albumin or Phospholipid
- No commercial melanin column is available for determining melanin affinity



Different compounds travel at different speeds in the chromatographic system. The differential migration depends on the lipophilicity and ionisation state.

$$k = \frac{\text{the number of moles in the stationary phase}}{\text{number of moles in the mobile phase}}$$

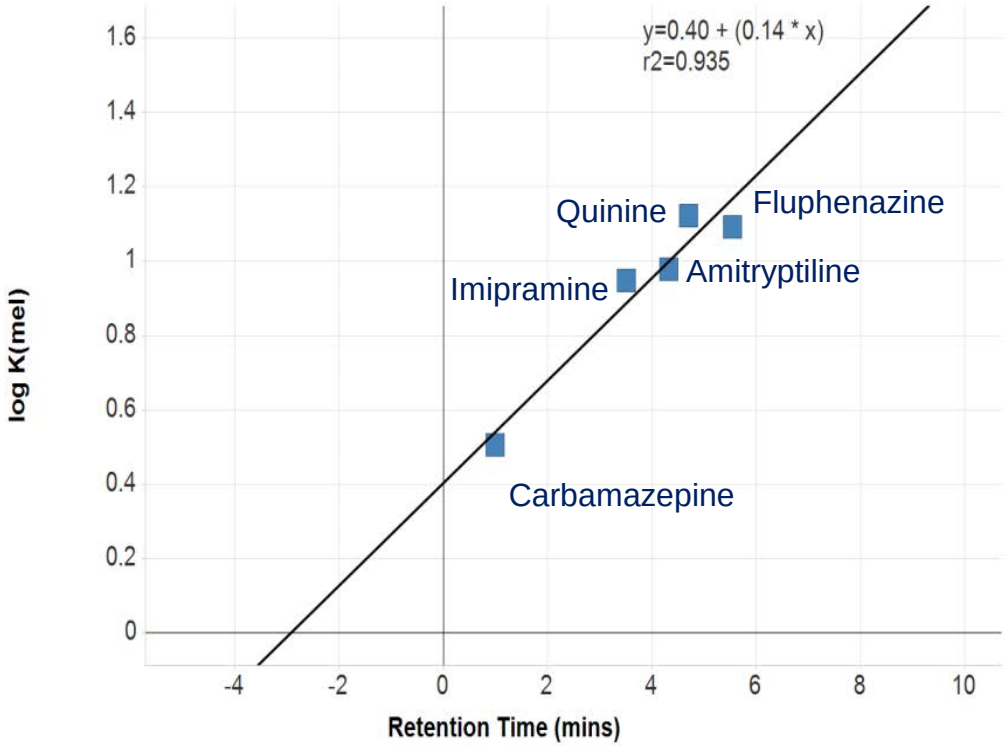


Adapted from Klara Valko's; J. Pharm.Sci. 92 (11) (2003) 2236-2248

NOVARTIS

Development of a melanin-affinity based in-vitro method

- Custom made melanin columns (50 x 3mm x 5um) based on published literature[1]
- Mobile phase: 0 - 30% IPA gradient ; (A) 50mM ammonium acetate buffer pH 7.4 (B) propan-2-ol
- Flow Rate: 1.0 ml min^{-1}
- High binders: Quinine, Fluphenazine, Amitriptyline, Imipramine,
- Low binder: Carbamazepine

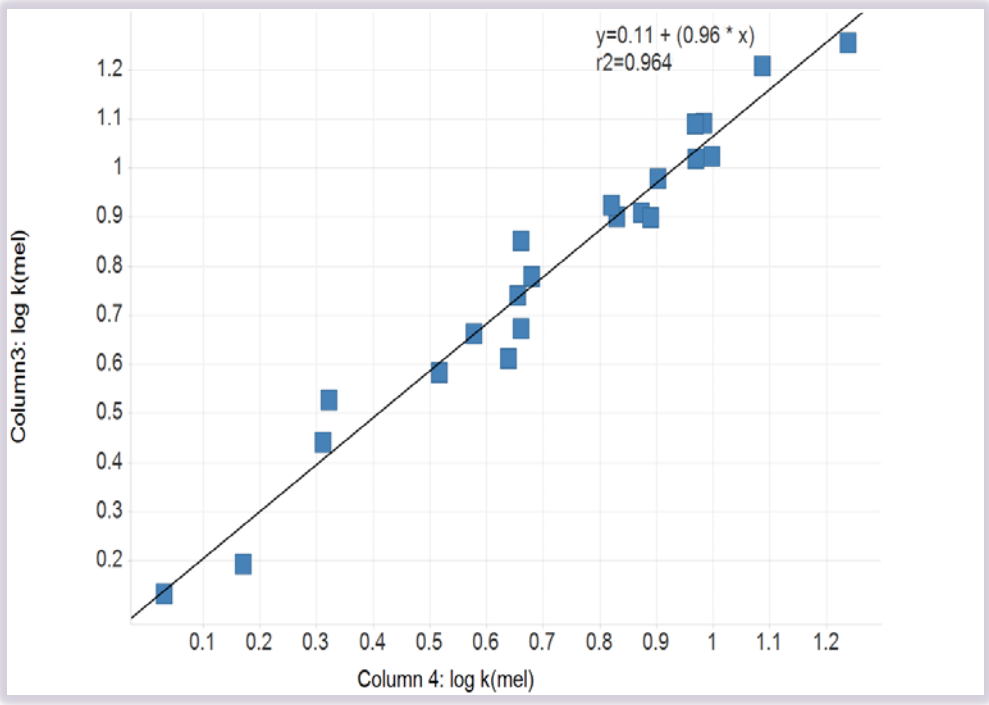


[1]*Ibrahim, H.; Aubry, A. Analytical Biochemistry 1995, 229, 272-277.*

NOVARTIS

Characterization of a chromatography based melanin affinity columns

Column to column variation appears to be acceptable

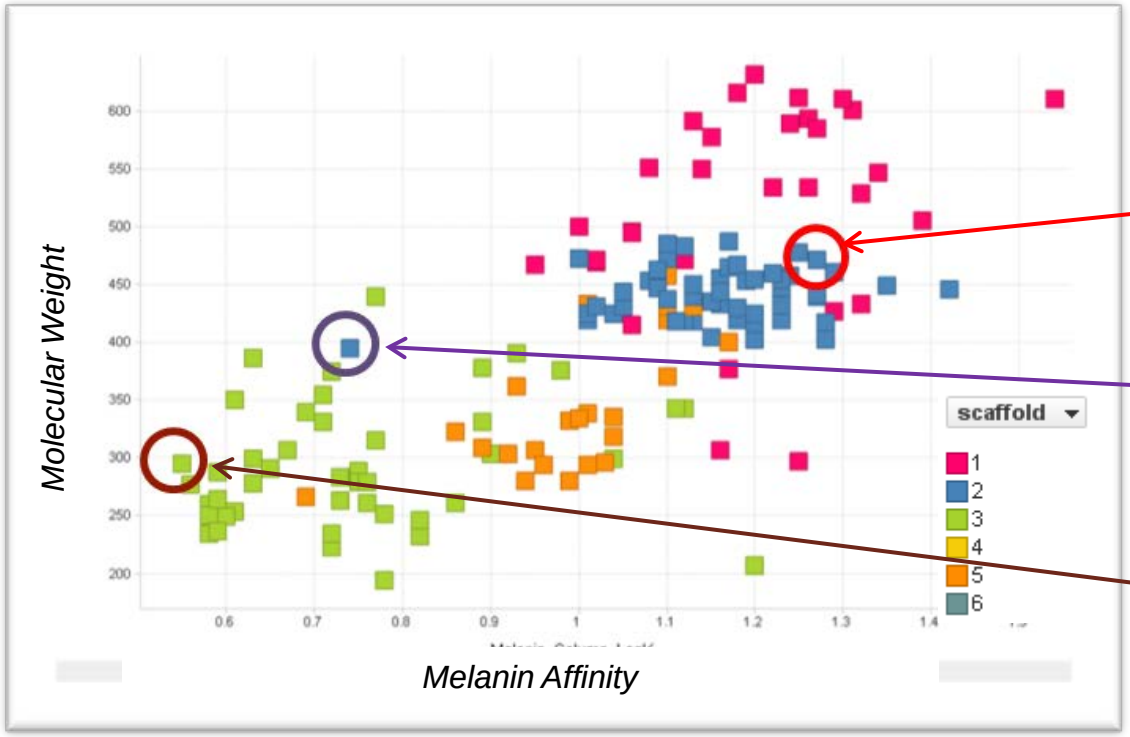


Chromatography based melanin column seems to be suitable for identifying trends

	Traditional method		LC method	
Compound	**Rank**	**CR1**	**LC rank**	**LogKmel**
NVS-1	1	0.67	2	0.84
NVS-2	2	1.55	1	0.69
NVS-3	3	5.50	4	1.18
NVS-4	4	8.45	3	1.05
NVS-5	5	18.33	6	1.42
NVS-6	6	62.62	5	1.24

NOVARTIS

Molecular scaffolds influence melanin binding affinity



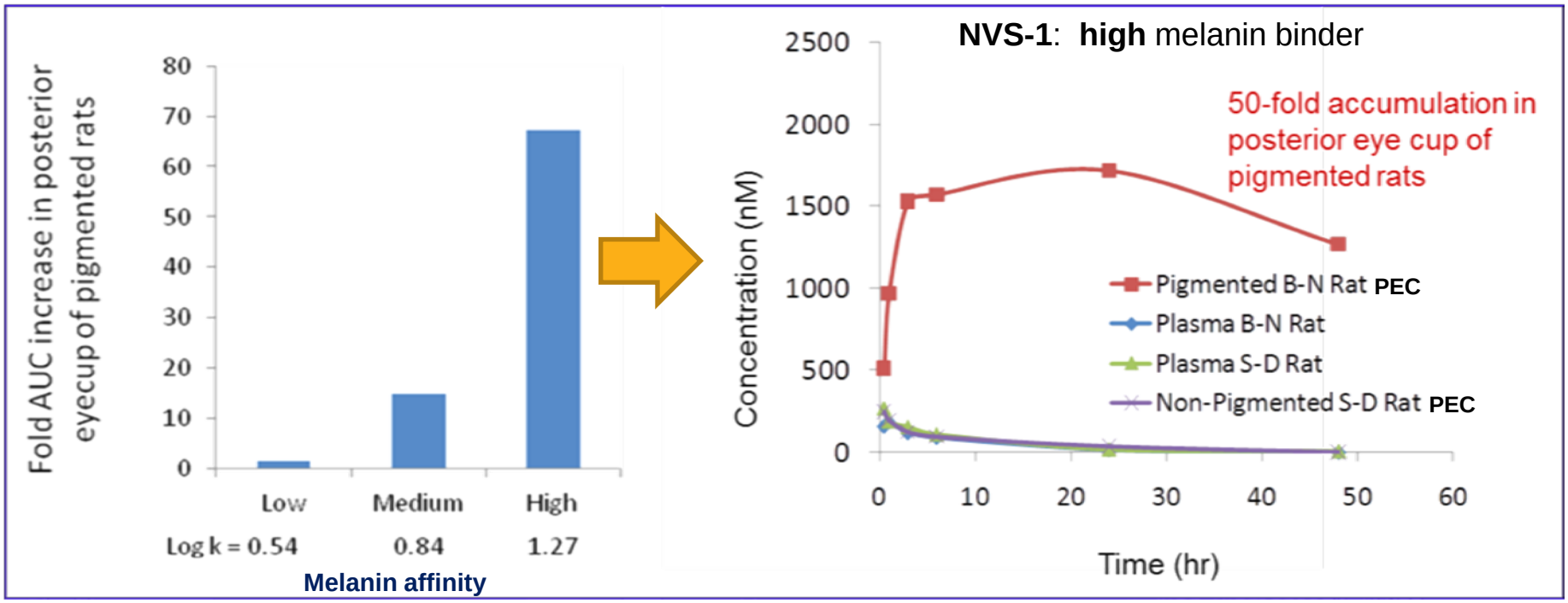
- 270 compounds have been screened in this high-throughput assay. This represents a larger and more diverse data set than existing literature data sets.

NOVARTIS

In vivo validation of the in-vitro affinity method via ocular pharmacokinetics

- **Objective** :
  - Establish a correlation between *in-vitro* and *in-vivo* assays
- **Study protocol:**
  - High affinity – NVS-1
  - Medium affinity – NVS-2
  - Low affinity – NVS-3
- **Strain/Route of Administration:**
  - Brown Norway (pigmented) and Sprague Dawley (non – pigmented) Rats
  - IV injection
- **Dose:** 1mpk solution (0.25mL)
- **Time Points**: 0, 30m, 1hr, 3hr, 6hr, 24hr, 48hr
- **Tissue Collected**: Retina, PEC, and Plasma
- **Bioanalysis** was performed by LC-MSMS

Significant increase in exposure in posterior eye cup of pigmented rats was observed for a high melanin binder



"high" melanin affinity (NVS-1):

- PEC exposure: Pigmented rats >> non-pigmented rats (~50x)
- Retina exposure: Pigmented rats > non-pigmented rats (~2x)
- No significant difference in plasma exposure between pigmented and non-pigmented rats

Time (hr)

Summary

- Melanin binding can impact ocular pharmacokinetics
- Validated a melanin affinity based in-vitro method
- Established *In vitro–in vivo* correlation (IVIVC)
- *In vitro* melanin binding assay can be used for **rank ordering** or differentiating the compound based on their ocular melanin affinity
- Future opportunities: C57/BL6 and B6(Cg)-Tyrc-2J/J (Tyrosinase deficient mice)

Applications to Drug Discovery and Development

- Evaluate drug-melanin binding characteristics at an early stage of drug discovery
  - *in vitro* assays
- If Melanin-binding is found, then check for reversibility and it's impact on ocular and plasma PK
  - *in vivo* assay in pigmented and non-pigmented animals
- If irreversible and high affinity drug-melanin is observed, run QWBA for drug distribution in skin, ear and brain (sensory organs)
- High melanin affinity compounds should be then discussed with PCS and Translation Medicine colleagues to enable them to modify the protocol if necessary
  - at least one pigmented species in toxicity studies
- Species and strain specific differences in melanin levels need to be considered during interpretation of preclinical data

Acknowledgements

- NIBR Ophthalmology/ Pharmacology team
  - Timothy Drew, Debby Long, Bruce Jaffee
- NIBR Chemistry team
  - John Reilly, Cornelia Forster, Mike Serrano-Wu
- NIBR MAP and Analytical Sciences team
  - Jakal Amin, Ann Brown, Vinayak Hosagrahara
- NIBR Computer Aided Drug Discovery team
  - Sarah Williams

NOVARTIS