What role do animal models play in developing next-generation melanoma therapies?
Melanocytes colour our hair, skin and eyes

- Melanocytes are skin cells that synthesize pigment and are the melanoma cell of origin
- Pigment protects us from the harmful effects of UV
- Lighter skin is found in regions of the world where there is less sunshine
Why are model systems important for melanoma research?
Why are model systems important for melanoma research?

- Fundamental biological processes shared in cellular life
- Discovery and function (test hypotheses)
- Disease genes are shared in mammals and vertebrates
- Ease of manipulation (genes, molecules, drugs)
- Large numbers, clonal
- Ethical implications
80% of disease genes shared with humans from vertebrates, fertilized at the one-cell stage. Hundreds of embryos from parent pair develop melanoma and...
The Zebrafish Life Cycle
How can zebrafish help discover new melanoma therapies?

Developmental lineages

Melanoma models
Fishing for new therapies

1. Zebrafish models of melanoma reveal new resistant cell populations
BRAF$^\text{V600E}$ mutations promote nevi

BRAF$^\text{V600E}$ melanoma model
Genomic Classification of Cutaneous Melanoma

Graphical Abstract

Identification of Genomic Subtypes
- **BRAF**
  - Patients younger
  - BRAF, MITF amplifications
- **RAS**
  - MAPK activation and AKT3 overexpression
- **NF1**
  - Patients older
  - Mutation burden
- **Triple wild-type**
  - Lacks UV signature
  - Copy number and complex rearrangements

Identification of Immune Signatures (independent of subtypes)
- mRNA immune signature
- LCK protein expression
- (left) Lymphocytic infiltration (LI) = improved survival

Clinical Management Implications
- **BRAF**
  - MAPK inhibitors
    - LI - 30%
- **RAS**
  - MAPK inhibitors
    - LI - 25%
- **NF1**
  - MAPK inhibitors
    - LI - 25%
- **Triple wild-type**
  - RTK inhibitors
    - LI - 40%
- Immunotherapies (mAb against immune checkpoints, high dose IL-2)

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In Brief
An integrative analysis of cutaneous melanomas establishes a framework for genomic classification into four subtypes that can guide clinical decision-making for targeted therapies. A subset of each of the genomic classes expresses considerable immune infiltration markers that are associated with improved survival, with potential implications for immunotherapy.
Temperature sensitive $mitfa^{vc7}$ allele: MITF-low to MITF-no activity.

Johnson et al., Dev Biol 2011
Taylor et al., Development 2011
Lister et al., JID 2015
MITF\text{Low} co-operates with p53 to promote melanoma (BRAF-independent)
BRAF$^{V600E}$ drives accelerates MITF$^{Low\, p53^{Mut}}$ melanoma onset and progression.

Superficial growth pattern

Unpigmented nodular

nodular

$Tg(mita:BRAF^{V600E})\; mita^{c7}p53^{M214K}$

![Graph showing tumor-free survival]

- BRAF-model (33/33)
- non-BRAF model (94/129)

$p < 0.0001$
MITF ON-OFF-ON : melanoma growth, regression and relapse
Single-cell sequencing of melanoma, residual disease and recurrence
Residual disease contains new cell states and cells states from the original tumor
Key Findings:

• Melanomas depend on MITF (their melanocyte identity) for survival, but some cells are independent and enable the melanoma to grow back.

Big Picture Concept:

Melanomas are comprised of subpopulations, with different survival potential.
1. Zebrafish models of melanoma reveal new resistant cell populations
2. Drugs that target stem-cell like populations in melanoma
Phenotypic drug screens in zebrafish

- New targetable pathways in melanocyte biology
- Gene-Drug screens to identify sensitizers of pigmentation
- New cancer target for an antibiotic
- Repurpose MEK inhibitors to treat CFC syndrome & anxiety
- Model and treat vascular malformations
- Models to test bioorthogonal chemistry

McCrae & Peterson, *Nature Drug Discovery* 2015
Phenotypic small molecule screens on the melanocyte lineage

Melanocyte Development
(Embryonic lineage)

Fewer melanocytes
5-Nitrofurans are anti-bacterial/parasitical pro-drugs

- 5-NFNs are pro-drugs
- Chagas disease: bioactivated by NTR
- Neuroblastoma: unknown
- Phase II clinical trial NCT00601003

Study of Nifurtimox to Treat Refractory or Relapsed Neuroblastoma or Medulloblastoma
Nifuroxazide targets ALDH$_{1}^{\text{High}}$ subpopulations in melanoma

Aldefluor Assay marks ALDH$_{1}^{\text{High}}$ cells

Nifuroxazide

ALDH$_{1}^{\text{ox}}$ (inactive)

* Nifuroxazide

Cell death
Nifuroxazide targets $\text{ALDH1A3}^{\text{High}}$ subpopulations in melanoma tumors
$ALDH1A3^{High}$ depleted subpopulations impact melanoma tumor initiation
**ALDH$$^\text{High}$$ cells are enriched in a patient samples following BRAF+MEKi treatment**

**Pre-BRAFi**  |  **Post-BRAFi**  
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![Image 1](image1.png)  | ![Image 2](image2.png)  
![Image 3](image3.png)  | ![Image 4](image4.png)  

Lynn Schuchter, Sana Sarvi, Yuting Lu
Key Findings:

• 5-Nitrofurans target and kill ALDH1\textsuperscript{High} tumour initiating cells in melanoma

• ALDH1\textsuperscript{High} cells are enriched in some patients following treatment

Big Picture Concepts:

• Cancer “stem-cells” can be targets

• Understanding the targets of drugs widens their potential for use in treatment of other diseases
Why are model systems important for melanoma research?

Basic (Foundational) Science

Model systems

Clinical Science

Translational science