**Motivation and Objectives**

**Motivation:** Myc dimerizes with Max to promote the transcription of genes associated with cellular proliferation, differentiation, and survival. Deregulation of Myc expression initiates and maintains approximately 30% of human cancers, making Myc an excellent target in oncology.

**Objective:** Develop potent inhibitors of Myc-Max dimerization that are suitable as an in vivo therapy.

**Challenge:** Myc-Max dimerization is difficult to inhibit due to the relatively large interface over which Myc-Max interactions occur. As a result, Myc has been considered “undruggable.”

**Hypothesis**

We hypothesize that epitope-targeted peptide ligands that adsorb at the Myc-Max interface will disrupt the interprotein interactions and prevent dimerization.

---

**Introduction**

- Myc-Max dimers recognize DNA and promote transcription
- Deregulation of Myc initiates and maintains nearly 30% of human cancers
- Large inter-protein interface makes Myc-Max dimerization difficult to inhibit

**Crystal Structure of Myc-Max Dimer**

PDB ID: 1NK5

**Epitope 1**

**Epitope 2**

**Epitope 3**

---

**Approach: Epitope-targeted Capture Agents**

- Typically yields ligands with high affinity (Kd of 100 pm – 10 uM) to target epitope
- Rapid sub-month development time
- Judiciously target three epitopes on Myc dimer interface

---

**In situ click screen to identify high-affinity ligands to the Myc dimer interface**

**Anti Screen: Improve Binding Selectivity**

**Scrambled Epitope 1**

**Scrambled Epitope 2**

**Scrambled Epitope 3**

**1. Streptavidin Alkaline Phosphatase**

**2. BCIP/NBT**

**Product Screen: Screen for ligands that bind to Myc Epitopes**

**Synthetic Epitope 1**

**Synthetic Epitope 2**

**Synthetic Epitope 3**

**Identify hit sequences by mass spectrometry**

**Example mass spectrometry analysis to sequence a peptide**

- Linear: 20%
- Cyclic: 80%

---

**Optical Images of Enzymatically-Developed Beads**

- Counterstain procedures yield different colored beads for anti-screen and product screens
- Some beads exhibit yellow color, which likely interferes with pre-clear
- Red and blue beads have unique fluorescence

---

**Faster Screening with Counterstaining Strategy**

**Motivation:** Eliminate need to manually remove beads from anti-screen (1 day to 2 weeks)

**Example Enzymatic Development Scheme**

**Counterstaining Approach**

**Optical Images**

- Yields different colored beads for anti-screen and product screens
- Red and blue beads have unique fluorescence

---

**Summary**

- Discovered 2 strong and ~20 medium peptide binders to Myc-Max dimer interface
- Counterstaining is a promising approach to accelerate screening for epitope-targeted peptides

**On-Going Work**

- Optimize counterstaining procedures
- Identify Myc ligand hits, with Bert Lai at Indi Molecular
- Synthesize ligands and test their efficacies to inhibit Myc-Max dimerization

---

**Conclusion**

**Acknowledgements**

I would like to thank Matthew Idso and Professor James Heath for their guidance and collaborative efforts throughout this process.

---

**Funding:**

**Collaborators:**

- Indi Molecular
- Mercy College
- Cal Poly
- CSU Systems Biology
- Institute for Systems Biology
- ImmPact
- Polyquick Fund
- USA National Institute of General Medical Sciences