

Ricardo Giordano, Ph.D.

Postdoctoral Fellow

Department of GU

Drs. Wadih Arap and Renata Pasqualini Laboratory

Identification of Therapeutic Targets for Leukemia by Phage Display Profiling of Leukemia Cell Lines and Patient-Derived Samples

Background:

Despite the advancements that have been made to the conventional systemic therapy of leukemia, drug resistance and non-specific cytotoxicity often result in adverse, long-term, side effects and significant relapse and mortality rates. In recent years, much attention has been focused on the development of targeted therapy for leukemia (Ravandi F et al., 2003). Recently, our group developed a new technology termed Biopanning and Rapid Analysis of Selective Interactive Ligands (BRASIL) that would allow for selective, single-cell targeting with genetically-modified phage libraries whereby filamentous phage display random peptides on their surface fused to the phage minor coat protein pIII. Our method comprises a simple procedure that allows cell-phage complexes to be separated from the remaining unbound phage in a single step (Giordano R et al., 2001). BRASIL has been successfully used to isolate phage that selectively targeted activated endothelial cells and tumor cells. In *ex vivo/in vivo* -based strategies, we have also used BRASIL to isolate phage that homes selectively to the bone marrow (unpublished data). We reasoned that profiling of leukemia cell lines and patient-derived samples would allow for the identification of novel therapeutic targets in leukemia.

Specific Aims:

- 1) Isolate peptide motifs (ligands) that preferentially bind to surface markers on leukemia cell lines and patient-derived samples.
- 2) Identify the corresponding receptors and assess the ligand – receptor pair interaction mechanisms.
- 3) Evaluate the lead pairs for the development of targeted therapy in leukemia.

Experimental Approach and Preliminary Results:

For specific aim 1, we used BRASIL to identify peptide sequences that preferentially bind to leukemia cells by incubating the cells with either the CX₅C or CX₇C random phage libraries, or with the insert-less control phage (Fd-tet). Phage bound to the cells was recovered, and quantified. BRASIL was performed on nine leukemia cell lines including MOLT-4, CCRF-CEM, SR, RPMI-8226, K-562, and HL-60 from the NCI-60 cell panel (<http://dtpws4.ncifcrf.gov>). Biopanning on MOLT-4 ALL cell line with CX₇C random phage library yielded several peptide motifs of which 3 clones exhibited high frequency binding to various leukemia cell lines and to bone marrow samples derived from 8 AML and 7 ALL patients.

By comparison of the selected motifs with available sequences in on-line protein databases, we identified a number of proteins (ligands) that share homologous sequences with these peptides. We then used biochemical methods to perform binding assays on a number of candidate receptors. Interestingly, relative to control insert-less phage, one of the clones showed a 52.27 ± 6.80 (average \pm SEM) – fold higher binding to recombinant neuropilin-1 (NRP-1), a receptor for VEGF₋₁₆₅, as compared to 1.90 \pm 0.67 – fold binding to an unrelated recombinant receptor ($P=0.011$).

Future Directions:

Since NRP-1 has been shown to play a role in angiogenesis and its overexpression has been linked to tumor progression and aggressiveness in many cancers including leukemia, work is underway to determine if the synthetic peptide exhibits similar strong binding to NRP-1 and whether it has any biological function on leukemia cells from cell lines and clinical samples. We will also perform binding and competition assays with synthetic and recombinant peptides to explore the ligand-receptor interactions. We will further validate the therapeutic efficacy of this peptide in mouse models of leukemia.

Significance:

These findings will have important clinical implications in that the newly identified peptide may serve as a peptidomimetic drug lead and can be optimized as a delivery vehicle for targeted therapy of leukemia. This approach has already proved to be fruitful through similar work published recently by our group (Zurita AJ et al., 2004).

References:

1. Ravandi F, Talpaz M, Estrov Z. Modulation of cellular signaling pathways: prospects for targeted therapy in hematological malignancies. *Clinical Cancer Research* 9: 535-550, 2003.
2. Giordano R, Cardó-Vila M, Lahdenranta J, Pasqualini R, Arap W. Biopanning and Rapid Analysis of Selective Interactive Ligands. *Nature Medicine* 11: 1249-1253, 2001.
3. Zurita AJ, Troncoso P, Cardó-Vila M, Logothetis CJ, Pasqualini R, Arap W. Combinatorial Screenings in Patients: The Interleukin-11 Receptor α as a Candidate Target in the Progression of Human Prostate Cancer. *Cancer Research* 64: 435-439, 2004.