Ligand-directed Therapy and Molecular Imaging Based on in vivo Phage Display Technology

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Our central working hypothesis is that differential protein expression in the human vascular endothelium associated with normal or diseased tissues offers the potential for developing novel diagnostic, imaging and therapeutic strategies. In essence, our research program uses combinatorial library selection (peptide- and antibody-based) to discover, validate, and exploit the vascular biochemical diversity of endothelial cell surfaces that can be subsequently exploited towards a new vascular-targeted pharmacology. Such targeting technologies may lead to the development of ligand-directed, clinically applicable agents for the treatment of patients. Translational applications, such as first-in-man clinical trials, which have now started within MDACC, will ultimately determine the value of this strategy. Indeed, the Food and Drug Administration (FDA) granted "safe-to-proceed" status for our first vascular-targeted IND in 2009. A first-in-human study has already been completed with several patients enrolled in this MDACC-sponsored clinical trial. A second IND was filed mid-November 2010; subsequent questions from the FDA were addressed though extensive additional experimentation, and the second Phase I study is now active. Two other drugs are in pre-IND stage, with several others in the pre-clinical laboratory phase. Long-term, the broader vision of our research is to generate a large-scale receptor map of the human vasculature. In this abstract, we present a summary of milestones and timelines as follows:

The Human Vascular Mapping Project

•2000: Scientific & ethical framework for human experimentation
•2002: Combinatorial screening in brain-dead and terminal wean patients (Arap et al. 2002)
•2004: Validation of the IL-11 receptor alpha as a target in prostate cancer (Zurita et al. 2004)
•2005: Intra- and inter-institutional ethics guidelines (Pentz et al. 2003, Pentz et al. 2005)
•2006: Design and validation of synchronous experimental approach (Kolonin et al. 2006)
•2008: Serial synchronous selection adapted for selection in patients (Staquicini et al. PNAS 2011)
•2008: IND filed for a Phase I clinical trial in prostate cancer patients phase (NCT00872157)
•2009: Translation of a targeted peptidomimetic (BMTP-11) in a first-in-man trial, Protocol No. 2008-0395 (Millikan et al. submitted)
•2010: Drug localization established in bone metastasis biopsies in 6 out of 6 patients treated
•2010: BMTP-11 licensing to Alvos Therapeutics, a new biotech company (subsidiary of Arrowhead Research)
•2010: Patient biopsy and autopsy material processed and captured on a data base with more than 2 MM ligands
•2011: Over 150 new vascular zip codes identified
•2012: Business development opportunities with Pharma leveraging normal and diseased tissue targeting
•2013: Additional screens in patients and Rhesus monkeys continue to build a database (millions of ligands targeting over 50 normal and diseased tissues)

DRUG DEVELOPMENT AND TECHNOLOGY TRANSFER

Our first IND (BMTP-11) received FDA "safe to proceed" status in Jan 2009. The first-in-man trial has been completed, having successfully met all target end points. A second Phase I trial using an anti-obesity drug candidate in cancer patients with high body mass is ongoing. Other IND applications focus on GRP-78 targeting for therapeutic and imaging purposes. Studies established the relevance of these targets in human cancer tissue samples from several tumor types evaluated. These studies indicate these targets are suitable for delivery of therapeutic and imaging agents. Efficacy in cancer has been validated in rodent models. Toxicology has been completed in mice and monkeys. We expect upcoming clinical studies to be informative and will establish a precedent for developing these targeted drugs. We generated a substantial patent portfolio for the Office of Technology & Commercialization. Table 1 depicts an update of our pipeline. Currently, we have intellectual property licensed to 5 biotech outfits, two of which are publicly traded.

Table 1. Internal Pipeline Ligand-directed Drugs Targeted to ZIP Codes: Update 2013

Name	*Drug Structure*	*Class*	*Phase*	*References*
BMTP-11	IL-11Rα targeted $_D$(KLAKKLAK)$_2$	Anti-cancer	IND	Arap et al. 2002, Zurita et al. 2004, Pasqualini et al. submitted
Arenegyr	CD-13 targeted hTNFα	Cytokine	IND	Arap et al. 1998, Ellerby et al. 1999, Corti et al. 2008
BMTP-78	GRP-78 targeted $_D$(KLAKKLAK)$_2$	Anti-cancer	Pre-IND	Mintz et al. 2003, Arap et al. 2004
Adipotide	Prohibitin targeted $_D$(KLAKKLAK)$_2$	Anti-obesity	IND	Kolonin et al. 2004, Barnhart et al. 2011
AGNP-14	Untargeted $_D$(KLAKKLAK)$_2$	Antibiotic	Pre-IND	Conley/Barbu et al. PNAS, 2013
$_D$(CLPRC)	VEGFR-1/Neuropilin-1 Sink	VTA	Lab	Giordano et al. Nat Med 2001, Chem Biol 2005, PNAS, 2010
$_D$(CARVC)	EGFR Molecular Decoy	Anti-cancer	Lab	Cardó-Vila et al. PNAS, 2010
2A2	Humanized Anti-lipid Rafts MoAb	Anti-XRT	Lab	Pasqualini and Arap 2004, Rotolo et al. J Clin Invest 2012

We developed an in vivo screening method in which peptides that home to specific vascular beds are selected after intravenous administration of a phage display random peptide library. This work has uncovered a vascular address system that allows organ-specific targeting to normal blood vessels and angiogenesis-related targeting to blood vessels of tumors. We also developed imaging technology to determine the distribution of such targeted probes in vivo, their organ-specificity and cellular receptors. Taken together, our previous work--among others--has suggested: (i) the vascular endothelium of organs is modified in a tissue-specific manner and (ii) the development of diseases with a vascular component is accompanied by specific abnormalities in the cells forming the blood vessels. A logical extension of our work is to isolate peptide motifs that home in vivo to the blood vessels of selective tissues affected by cardiovascular and lung disorders. This application represents the first step in this direction. Vast precedence indicates that phage can be safely administered to humans. Bacteriophage were discovered in the early 1900s, and their genomes were sequenced almost two decades ago. Phage were used for bacterial prophylaxis, cholera, and burns in the pre-antibiotic era. Phage have long been administered to pediatric patients to study their immune response. These data show that phage can be safely given to humans. The potential of in vivo phage display to identify targeting sequences has been largely explored by our group. We have used a large array of technologies and tested several types of libraries and experimental strategies and will expand on the existing receptor-ligand systems identified by phage library selection systems for targeted delivery. The integration of phage display-based combinatorial tissue targeting and nanotechnology has emerged from methodology we established for the direct-assembly of gold (Au) nanoparticles onto phage. Given the challenges for reproducibly building at the nanometer scale, including the need for streamlined and “bottom-up” approaches for assembling nanoparticle architectures, the combination of phage and Au nanoparticles is an ideal system to gather the required knowledge and understanding to control the fabrication and application of biologically assisted nano-assemblies. This work was motivated from the premise that, in nature, the direct-assembly of molecules and particles is often directed by non-specific hydrophobic, van der Walls, and/or electrostatic interactions. We hypothesized that the assembly of phage and Au nanoparticles may also occur spontaneously through similar interactions. The outcome from testing this hypothesis was the design and validation of a method for Au-phage-based nano-assembly without genetic manipulation or complex conjugation chemistry. We generated stable and biologically active networks of direct-assembled Au-phage scaffolds, in which we can fine-tune the chemical and physical properties of these biological structures. This tuning capability combined with the programmed tissue targeting property of the phage has allowed us to integrate multiple functions into a single nano-assembly, and thus serves as a complementary and non-mutually exclusive tool to use in diverse applications such as near infrared (NIR) surface enhanced Raman scattering (SERS) detection, Au enhanced fluorescence imaging and/or heat deposition for NIR photo-therapy. The identification of vascular markers targeted by circulating ligands will shed light on the complex cellular and molecular diversity of cardiovascular and lung diseases. We will validate the selected phage and peptide probes as delivery vehicles for targeted therapies and/or Au-phage based imaging agents. Our efforts will accelerate the development of novel therapies based on targeted delivery of biologically-based and -targeted nanoscaffolds. In the future, the integration of the molecular diversity of blood vessels with nanotechnology will be translated into clinical applications.