

First Look

The Next Wave of GCT Breakthroughs
in Health Care

2021

WORLD MEDICAL
INNOVATION
F O • R U M™



Mass General Brigham

Innovation

Harvard Medical School investigators kick-off the 2021 World Medical Innovation Forum with rapid fire presentations of their high-potential new technologies.

Eighteen rising stars from Brigham and Women's Hospital, Massachusetts General Hospital, Massachusetts Eye and Ear Infirmary and McLean Hospital will give ten-minute presentations highlighting their discoveries and insights that will disrupt the field of gene and cell therapy. This session is designed for investors, leaders, donors, entrepreneurs, investigators and others who share a passion for identifying emerging high-impact technologies.

2021 Gene and Cell Therapy Investigators

- 4 Natalie Artzi, PhD
- 5 Xandra Breakefield, PhD
Koen Breyne, PhD
- 6 Bob Carter, MD, PhD
- 7 Nino Chiocca, MD, PhD
- 8 Merit Cudkowicz, MD
- 9 Florian Eichler, MD
- 10 Anna Greka, MD, PhD
- 11 Penelope Hallett, PhD
- 12 J. Keith Joung, MD, PhD
- 13 Jeannie Lee, MD, PhD
- 14 Casey Maguire, PhD
- 15 Marcela Maus, MD, PhD
- 16 Patricia Musolino, MD, PhD
- 17 Pierpaolo Peruzzi, MD, PhD
- 18 David Scadden, MD
- 19 Christine Seidman, MD
- 20 Khalid Shah, PhD
- 21 Luk Vandenberghe, PhD

Affiliations

BWH | Brigham and Women's Hospital
MGH | Massachusetts General Hospital
HMS | Harvard Medical School
MEE | Mass. Eye and Ear
McLean

Versatile Polymer-Based Nanocarriers for Targeted Therapy and Immunomodulation



Natalie Artzi, PhD

Assistant Professor, BWH

nartzi@bwh.harvard.edu

The power of leveraging the patient's own immune system to fight diseases is increasingly being recognized, providing new opportunities for intervention. However, targeting the right tissue, and the right cell, at the right time becomes paramount. Biomaterials, such as nanoparticles, hydrogels, and medical devices, were originally designed as inert prosthetic materials, as depot for the embedment of cells, or to reduce the toxicity of drugs. Recently, materials have been demonstrated to be able to do so much more. Millions of people from all over the world have witnessed the wonders of liposome-based nanoparticles delivering mRNA-based COVID-19 vaccine. Indeed, in the absence of a proper delivery vehicle, the most potent drug becomes moot. This talk will explore emerging medical technologies that harness lessons from biomaterial development for drug delivery systems to those that will reprogram the immune system to generate robust and long-lasting curative outcomes in a range of diseases. I will provide examples of synthetic nanoparticles for gene therapy my lab has developed that afford high in vivo transfection efficiency for cancer combination immunotherapy. I will highlight new opportunities for continual innovation that can impact cancer, autoimmune diseases, and tissue regeneration therapies. The tools are at our fingertips.



Figure 1

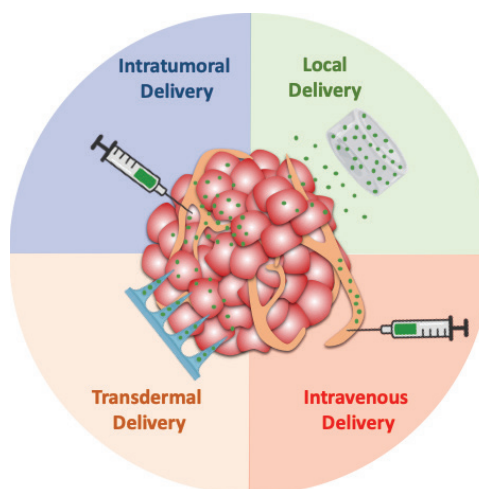


Figure 2

Enhancing Vesicles for Therapeutic Delivery of Bioproducts



Xandra Breakefield, PhD

Geneticist, MGH; Professor, Neurology, HMS
breakefield@helix.mgh.harvard.edu

Koen Breyne, PhD

Molecular Biologist, MGH; Instructor, Neurology, HMS
kbreyne@mgh.harvard.edu

Extracellular vesicles (EVs) are one of the most promising payload carriers for therapeutic interventions. These nanosized bioparticles comprise a protective membrane layer that shields their luminal cargo. They are secreted from virtually every living cell and are omnipresent in all biological specimens. EVs are being produced for therapeutic purposes by universal EV donor cells, such as mesenchymal stromal cells, and patient-derived cells.

Our aim is to develop a versatile modality for loading EV cargo for adoptive transfer therapies and thereby take advantage of the relatively large and multi-component cargo capacity of EVs, and their low immunogenicity and toxicity.

Proteins with a highly positive net charge can associate with and/or diffuse through biologic membranes similar to membrane penetrating peptides. When these supercharged proteins are mixed with isolated EVs *ex vivo*, they are taken up into EVs, then termed supercharged EVs (scEVs). Single EV resolution techniques assess our loading efficiency with scEVs at approximately 10% of the EVs in suspension with about 1 to 30 molecules per vesicle. The positive charge of the protein used to generate scEVs has an added advantage that it can associate with and piggyback negatively charged agents, such as RNA or DNA. We have shown that loading biocargo into scEVs improves their ability, over natural EVs, to deliver functional cargo into the cytoplasm of recipient cells. The flexibility of combining different cargo species into scEVs opens up new approaches for cutting-edge technologies, such as gene editing where recombinant Cas9, sgRNAs and HDR DNA molecules can be delivered together in scEVs, and will biodegrade after gene editing has occurred in the recipient cells.

In summary, our scEV delivery technology is a universal technique that is applicable to functional delivery of multiple biomolecules, such as protein and RNA/DNA cargo into recipient cells in culture and *in vivo*.

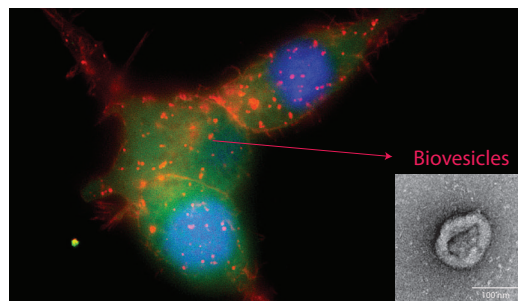


Figure 1. Nanoscaled biovesicles are secreted by virtually any cell and can be used as a carrier for therapeutic cargo.

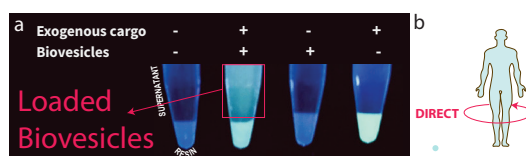


Figure 2. Synthetic reprogramming of biomolecules enable exogenous loading of cell-derived biovesicles (a). These methods can be applied to any vesicle freely available in biofluids of patients circumventing immunogenicity problems observed with other delivery vectors.



Personalized iPSC-Derived Dopamine Progenitor Cells for Parkinson's Disease

Bob Carter, MD, PhD

Chairman, Department of Neurosurgery, MGH; William and Elizabeth Sweet Professor of Neurosurgery, HMS
bcarter@mgh.harvard.edu

We describe the development of a strategy or the implantation of patient-derived midbrain dopaminergic progenitor cells, differentiated in vitro from autologous induced pluripotent stem cells (iPSCs), and provide an example of the treatment of a patient with idiopathic Parkinson's disease. Supported by extensive pre-clinical data, and technical advances in iPSC generation and dopaminergic precursor prediction, we generate well characterized population of patient-specific progenitor cells. These are produced under Good Manufacturing Practice conditions and are characterized as having the phenotypic properties of substantia nigra pars compacta neurons; testing in a humanized mouse model (involving peripheral-blood mononuclear cells) indicated an absence of immunogenicity to these cells. Cells are implanted into the putamen (left hemisphere followed by right hemisphere, 6 months apart) without the need for immunosuppression. Positron-emission tomography with the use of fluorine-18-L-dihydroxyphenylalanine suggested graft survival. An example of this strategy will be presented, including the description of clinical measures of symptoms of Parkinson's disease after surgery, which stabilized or improved 24 months after implantation.

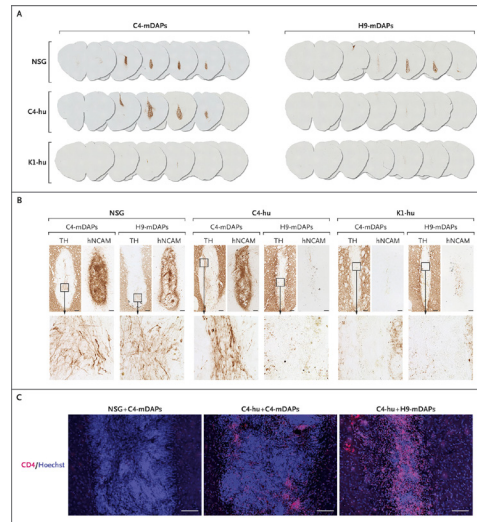


Figure 1. Immunogenicity of Midbrain Dopaminergic Progenitor Cells (mDAPs) in Humanized Mice.

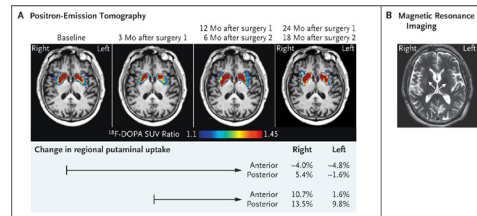


Figure 2. Axial fluorine-18-L-dihydroxyphenylalanine (18F-DOPA) positron-emission tomographic images at the level of the basal ganglia at the time points indicated.



Oncolytic Viruses: Turning Pathogens into Anticancer Agents

Nino Chiocca, MD, PhD

Neurosurgeon-in-Chief and Chairman,
Neurosurgery, BWH; Harvey W. Cushing
Professor of Neurosurgery, HMS
eachiocca@partners.org

Several solid tumors remain impervious to therapies, such as targeted drugs or immunotherapy. Amongst these tumors are the malignant brain cancer, glioblastoma (GBM), but there are also others, like pancreatic cancer. Two reasons for treatment resistance are the profound heterogeneity of aberrant cancer signaling pathways within cells of each tumor and the relative absence of antitumor compared to the abundance of protumor immune cells within the tumor microenvironment.

Oncolytic viruses (OVs) are either naturally derived strains or genetically engineered variants of viruses that target tumor cells specifically via infection and/or tumor-selective replication to generate progeny OVs in tumors. These progeny OVs can then go onto infect surrounding tumor cells. The processes of infection and replication generate a wave of propagating OVs throughout the tumor. During this, immunogenic factors and tumor cell death lead to activation of innate immune cells, such as neutrophils, macrophages, and ultimately activation of cytotoxic T cells (**Figure 1**). These will scour the tumor to attack initially antigens from the attacking OV but the infiltrating T cells become “educated” about the tumor antigens in the tumor microenvironment and start to attack these too, thereby setting up a vaccination process against the tumor. OVs can provide an answer to the problem of tumor cell heterogeneity since the administered therapeutic virus only needs to infect a few cells and then this will lead the patient’s own immune cells to become educated over time on the heterogeneous nature of the tumor “antigenome”. They also provide an answer to the problem of immunosuppressive nature of the tumor microenvironment since the ongoing tumor-specific viral infection changes the makeup of the immune cells from a pro-tumor to an anti-tumor phenotype. In common parlance, OVs turn tumors from immunologically “cold” to “hot” (**Figure 2**).

Our OVs can be injected in GBM cancers in human brains. In a phase 1 clinical trial in humans, we can show that OV and its antigens lead to tumor death but also can persist for several months. This does lead to persistent infiltration of cytotoxic T cells in tumor (**Figure 3**). There is also expansion of T cell clones in patient’s blood shared with T cells in patient’s tumors, the first step of an immunological process needed for tumor rejection.

Therefore, OVs are safe to administer in humans even in the brain, can persist within tumor, and can increase immune cell infiltration and recognition of tumor antigens. Pursuit of these OV technologies in both early and late phase clinical trials of solid cancer either with academic and/or private funding remains the necessary step for commercialization.

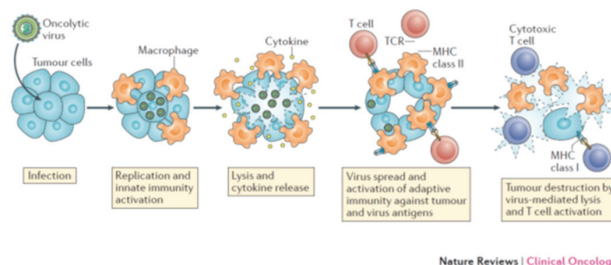


Figure 1

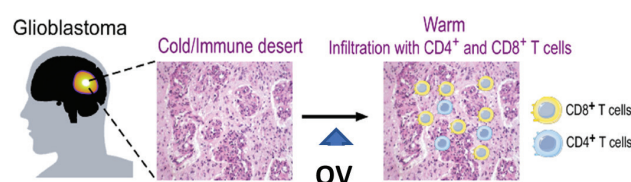


Figure 2. OV turns tumors from immune “cold” to “hot”

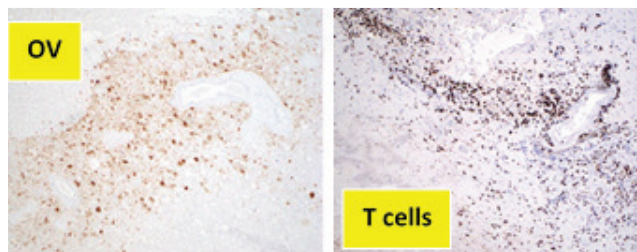


Figure 3. Human tumors injected with OV show persistence of OV and T cell infiltrates

Gene Therapies for Neurological Disorders: Insights from Motor Neuron Disorders



Merit Cudkowicz, MD

Chief of Neurology, MGH

cudkowicz.merit@mgh.harvard.edu

We are an unprecedented time in therapeutics for both genetic and non-genetic forms of neurological disorders. Technologies to lower or raise levels of specific proteins in the spinal cord and brains in people, are leading investigators and pharma to develop treatments for many serious disorders that lack treatments. Recent Examples include antisense oligonucleotide and AAV-mediated approaches in spinal muscular dystrophy and familial amyotrophic lateral sclerosis. The ability to use these same technologies to target proteins important for broader sporadic forms of neurodegenerative disorders are already underway.

Using examples from motor neuron disorders, I will discuss different gene therapy approaches in human trials and in development.

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Rare but Mighty: Scaling Up Success in Single Gene Disorders



Florian Eichler, MD

Director, Center for Rare Neurological Diseases, MGH;
Associate Professor, Neurology, HMS

feichler@partners.org

The Eichler laboratory studies fundamental aspects of lipid metabolism in relation to neurodegeneration of human single gene disorders. The lab combines a cell and animal biology focused program with tools of lipid analysis, vector biology and gene editing. While distinct human mutations consistently impact various species of phospholipids and sphingolipids, the consequences on individual cell types and the overall function of the nervous system vary. By analyzing lipids in relation to neuronal function, we aim to improve cell targeting and biodistribution of gene correction strategies entering the clinic (lentiviral, AAV-mediated, gene editing), while minimizing potential toxicity. Specific human mutations affecting serine palmitoyl transferase result in distinct lipid signatures that affect sensory and motor neurons differently. Recently we uncovered the mechanism by which sphingolipid synthesis escapes regulation, thereby affecting early myelination and neuronal development. Similarly, for phospholipid metabolism we are learning how mutations in *ABCD1* affect neurotransmitters, explaining selective vulnerability associated with altered lipid chemistry. The study of regulatory mechanisms underlying neuronal subtypes, synaptic plasticity and interactions with glia are now a major focus of the laboratory. Concurrent biomarker development emerging from these insights allows us to rapidly scale up success in trials of single gene disorders.

Unlocking the Secret Lives of Proteins in Health and Disease



Anna Greka, MD, PhD

Associate Professor, Medicine, BWH;

Associate Professor, Medicine, HMS

agreka@bwh.harvard.edu

The mission of the Greka laboratory is to define fundamental aspects of membrane protein biology and dissect mechanisms of cellular homeostasis. The laboratory complements this cell biology-focused program with tools from molecular biology, genomics, proteomics, and chemical biology.

Combining expertise in ion channel biology with the study of kidney podocytes, the Greka laboratory uncovered a pathway linking TRPC5 ion channel activity to cytoskeletal dysregulation and cell death. Based on these discoveries, TRPC5 inhibitors are now being tested in the clinic for difficult-to-treat kidney diseases.

More recently, the Greka laboratory made a key discovery of a general mechanism that monitors the quality of membrane protein cargoes destined for the cell surface by studying a proteinopathy in the kidney, caused by a mutation in MUC1. Specifically, the Greka lab identified a mechanism for membrane protein quality control that is operative in diverse cell types and tissues, such as kidney epithelial cells and retina photoreceptors. The study of cargo quality control is now a major focus of the laboratory.

The Greka laboratory is also interested in dissecting the fundamental mechanisms of cellular homeostasis across the lifespan, with implications for many degenerative human diseases.

New Scientific and Clinical Developments for Autologous Stem Cell Therapy for Parkinson's Disease Patients



Penelope Hallett, PhD

NRL, McLean; Assistant Professor of Psychiatry, HMS
phallett@mclean.harvard.edu

Degeneration of midbrain dopamine neurons and subsequent loss of functional dopamine synapses in the caudate-putamen causes the primary motor symptoms in Parkinson's disease. Current drug and experimental gene therapies attempt to enhance the function of the remaining 20-30% of these dopaminergic synapses, however, such dopaminergic pharmacological enhancement do not work long term and produce side-effects. The replacement of the patient's own dopamine cell and the repopulation of synapses in the caudate-putamen is envisioned as a permanent regenerative medicine solution for motor symptoms in PD ([1][2]) (**Fig.1**).

With over two decades of careful biological and mechanistic studies of cell therapy models and pioneering work on cell therapy for PD, we have arrived at an optimized cell source for cell therapy in PD, which is induced pluripotent stem cell (iPSC)-derived autologous midbrain dopamine neurons ([3][4][5]) (**Fig.2**). We have carried out research that has now reached a late-stage preclinical phase, including evidence in non-human primates with parkinsonism that autologous cells can repopulate the lost dopaminergic synapses, restore function and reduce symptoms (**Fig.3**).

This presentation will show our data from current scientific and research efforts establishing how reprogrammed human peripheral blood monocytes can be used in clinical trials planned over the next few years.

References:

- [1] Mendez I et al Isacson O. Cell type analysis of functional fetal dopamine cell suspension transplants in the striatum and substantia nigra of patients with Parkinson's disease. *Brain*. 2005;128:1498-510.
- [2] Hallett PJ et al Isacson O. Long-term health of dopaminergic neuron transplants in Parkinson's disease patients. *Cell Rep*. 2014;7:1755-61.
- [3] Hargus G et al Isacson O. Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats. *PNAS*. 2010;107(36):15921-6.
- [4] Hallett PJ et al Isacson O. Successful function of autologous iPSC-derived dopamine neurons following transplantation in a non-human primate model of Parkinson's disease. *Cell Stem Cell*. 2015; 16:269-74.
- [5] Osborn TM et al Isacson O. Advantages and Recent Developments of Autologous Cell Therapy for Parkinson's Disease Patients. *Front Cell Neurosci*. 2020;14:58.

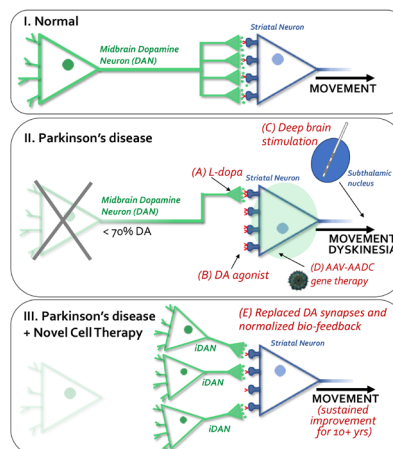


Figure 1. Overview of available or experimental treatment modalities for Parkinson's disease

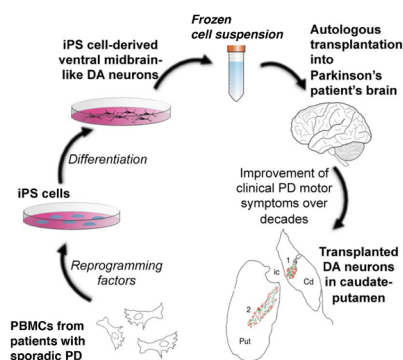


Figure 2. Using iPSCs as a cell therapy source for autologous transplantation

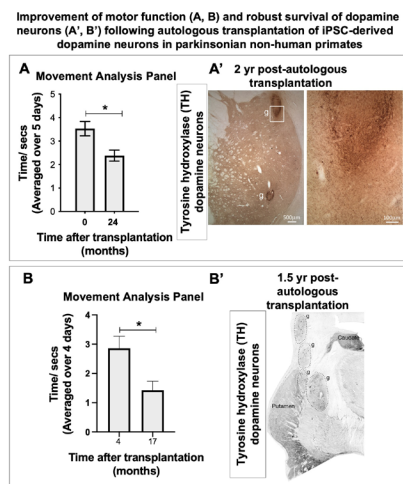


Figure 3. Autologous stem cell therapy improves parkinsonism in pre-clinical models



Treating Rett Syndrome through X-Reactivation

Jeannie Lee, MD, PhD

Molecular Biologist, MGH; Professor of Genetics, HMS

lee@molbio.mgh.harvard.edu

The X-chromosome harbors hundreds of disease genes. Among them is *MECP2* whose mutation results in Rett Syndrome, a severe neurodevelopmental disorder for which there is currently no disease-specific treatment. Because female cells carry two X-chromosomes, an emerging treatment strategy has been to reawaken the healthy allele on the inactive X (Xi). Mouse models suggest that as little as 5-10% *MECP2* restoration improves neuromotor function and extends lifespan by 5- to 8-fold. Through a comprehensive screen, we have identified several preclinical candidates that can induce partial Xi-reactivation. Combining an XIST antisense oligonucleotide and a small molecule inhibitor of DNA methylation results in an impressive pharmacological synergy, whereas neither agent alone achieves reactivation. The drug combination leads to *MECP2* upregulation in the mouse brain and also to Xi-reactivation in patient cell lines. To investigate safety of selective Xi-reactivation, we tested genetic models and examined consequences in different mouse organs. There was a surprising tolerance to Xi-reactivation in various organs. Thus, selective Xi-reactivation may be an effective and well-tolerated approach to treat X-linked neurodevelopmental disorders such as Rett Syndrome.

Enhanced Gene Delivery and Immuno-evasion of AAV Vectors without Capsid Modification



Casey Maguire, PhD

Associate Professor of Neurology, MGH & HMS

cmaguire@mgh.harvard.edu

In vivo gene therapy with adeno-associated virus (AAV) vectors has advanced to two FDA-approved medicines, with many more therapies progressing through clinical trials. Virus-based delivery systems have paved the way in human gene therapy, owing to their relatively high efficiency of delivery. That said, the human body recognizes viruses such as AAV as foreign, which can initiate immune responses that can lead to dose-limiting toxicities in some cases, and completely prevent dosing of a subset of patients with pre-existing circulating anti-AAV antibodies. Further, efficiency of gene delivery to particular organs still remains quite low. Finally, if transgene expression wanes over time, it is not possible to redose AAV due to the immune response against the initial injection. Strategies to overcome these hurdles include capsid engineering to evade antibodies and increase efficiency, and immunosuppressive drugs to reduce the immune response. None of these strategies completely addresses all of these issues. Also, capsid engineering often confers an unpredictable capsid biodistribution and transgene expression.

Approximately a decade ago, we made the striking observation that during AAV vector production, a portion of the capsid becomes encapsulated in extracellular vesicles (EVs), essentially becoming an enveloped AAV, eAAV. We initially hypothesized that the envelope on eAAV would confer unique properties including protection from anti-AAV antibodies and enhanced transgene delivery, and which we and others subsequently demonstrated in published work in preclinical mouse models. We are now intensely focused on developing the eAAV technology for clinical application, which requires efficient production and purification methods. Recent discoveries seem to suggest that AAV may have evolved to direct some of its capsids to be encapsulated in host cell-derived membranes, which should provide targets to improve and develop the system for use in human gene therapy.

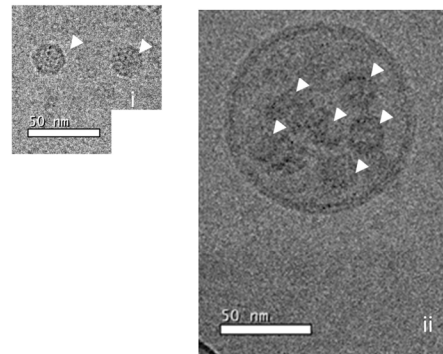


Figure 1. Conventional AAV (i) and enveloped AAV (ii). Arrows point to capsids.

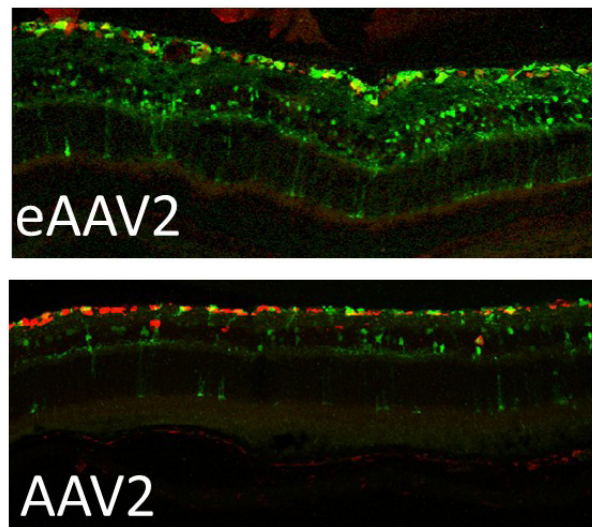


Figure 2. GFP expression from eAAV2 or AAV2 after intravitreal injection in mice.



Cell Therapy Innovations at MGH

Marcela Maus, MD, PhD

Director, Cellular Immunotherapy, Cancer Center, MGH; Associate Professor of Medicine, HMS
mvmaus@mgh.harvard.edu

Gene-modified T cell therapy has emerged as transformative therapy for B cell leukemia, B cell lymphoma, and multiple myeloma. However, there some patients relapse with tumors that have lost expression of the target molecule, or have lost persistence of their T cells before there is complete eradication of their tumor. Next-generation strategies will require targeting more than one molecule in B cell malignancies. In addition, there is still untapped potential for gene-modified T cell therapy in blood cancers derived from myeloid cells and T cells, and for solid tumors. The Cellular Immunotherapy Program encompasses clinical development efforts and basic research labs at the Mass General Hospital Cancer Center. Our clinical team has played a prominent role in the development of CAR T cells for lymphoma and multiple myeloma. In the research lab, we have developed a variety of chimeric antigen receptors (CARs) targeting novel antigens in combination, using different technological strategies, each tailored for the respective obstacles in five different clinical diseases: B-cell leukemia, B-cell lymphoma, T-cell lymphoma, multiple myeloma, and glioblastoma. We will discuss our portfolio of pre-clinical and clinical-stage assets and our vision for their successful transition to the next phase of development.

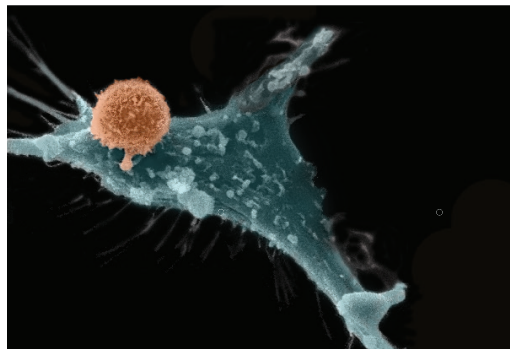


Figure 1

Translational CAR T Pipeline			
Design	Graphic Design	Target	Indication
CAR directed to new target		CD37	B & T cell lymphoma
Dual-specific tandem CAR		CD79b and CD19	B cell lymphoma
Dual-specific multimeric CAR		BCMA and TACI	Multiple Myeloma
CART secreting T-cell Engager Antibody Molecule		EGFRvIII and EGFR; others	Glioblastoma, others
Drug-controlled CAR		CD19; others	Acute Lymphocytic Leukemia, others

Figure 2



Gene Therapy for Cerebral Genetic Vasculopathies

Patricia Musolino, MD, PhD

Co-Director Pediatric Cerebrovascular Program, MGH;
Assistant Professor of Neurology, HMS

mmcdonnell@bwh.harvard.edu

Genetic vasculopathies altering vascular smooth muscle cell (VSMC) function can lead to progressive white matter degeneration and recurrent strokes. A common pathomechanism underlying cerebral ischemia is progressive narrowing of the cerebral vessels –intima hyperplasia- caused by VSMCs proliferation. Among the most severe forms of these steno-occlusive vasculopathies (moyamoya-like diseases) arise from missense mutations in the *ACTA2* gene where patients suffer white matter injury and recurrent arterial ischemic strokes during early childhood.

Using an *ex vivo* gene therapy approach and novel MR imaging biomarkers we learned that corrected autologous hematopoietic stem cells can restore white matter microvascular function in kids suffering white matter degeneration in X-linked Adrenoleukodystrophy. However, the need for myeloablative chemotherapy needed during cell transplantation and lack of cell specificity limits its translation to other genetic vasculopathies.

We, thus, set up to develop *in vivo* gene therapy to prevent steno-occlusive vascular disease using novel AAV vectors that can target endothelium and smooth muscle cells to deliver customized CRISPR prime editors under the control of VSMC disease-specific promoters to correct *ACTA2* missense mutations. Our preliminary work editing *ACTA2* mutations in patient-derived cells shows high efficiency on restoring sequence and functional phenotype with no significant off target effects. Leveraging our conditional knock in mouse model that recapitulates the human cerebrovascular disease we aim to complete our pre-clinical proof of concept needed for the first in human gene therapy for VSMC proliferative vasculopathies.

De-risking *in vivo* genetic modulation of VSMCs launches a platform to treat intima hyperplasia in non-genetic forms of cardiovascular disease.

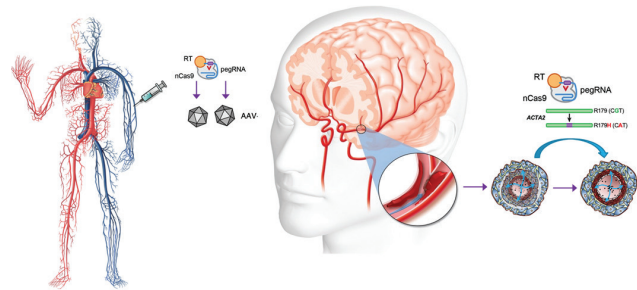


Figure 1. Schematic representation of gene therapy approach for SMC vasculopathies using systemic AAV injection to deliver CRISPR prime editor to correct single nucleotide of mutant allele and reverse intima hyperplasia in cerebral arteries.



RNA Therapy for Brain Cancer

Pierpaolo Peruzzi, MD, PhD

Neurosurgery, BWH; Assistant Professor of
Neurosurgery, HMS

pperuzzi@bwh.harvard.edu

MicroRNAs have been recognized in recent years as major players in almost any human diseases, but their therapeutic implications have been negligible, especially in cancer.

In our studies on glioblastoma, the deadliest brain tumor, we found that the value of microRNAs lies in numbers, as they work in groups, or clusters, some of which are simultaneously upregulated, while others downregulated. It is this combination of gains and losses that mostly affects the tumor's biology.

This has practical implications, since microRNAs offer an ideal genetic platform for artificially recapitulating this clustering landscape, and, additionally, to support expanded gene therapy solutions.

In nature, microRNAs are initially produced as long, primary RNA transcripts which are then cleaved by a processing enzyme into the biologically active, ~70 nucleotide-short microRNA "hairpins".

Since recognition of the hairpin structure is a fundamental step in this activation process, we have developed protocols to design and produce RNA-coding genes containing several hairpins of choice in tandem.

These hairpins are recognized and cleaved by the processor, and thus released in their active forms, recapitulating "clusters" of active microRNAs.

Additionally, the primary RNA can also be endowed with other specific sequences strategically located among the hairpins, so that they are also released upon cleavage.

We can thus design and synthesize "RNA superdrugs" which hitchhike the microRNA processing machinery of the cells and are cleaved into multiple active RNAs, including microRNAs, long non-coding RNAs which function as sponges for oncogenic microRNAs, and other non-coding RNAs which can be used to target specific oncogenes in the forms of RNA aptamers. The net result is a significant biological synergism among the different components of the transgene.

This gene therapy platform is highly versatile and independent of the microRNAs or ncRNAs of choice. Also, the small size of the primary RNA allows it to be fitted into any delivery vectors currently used for gene therapy, and makes it also suitable for systemic or loco-regional delivery via extracellular vesicles.

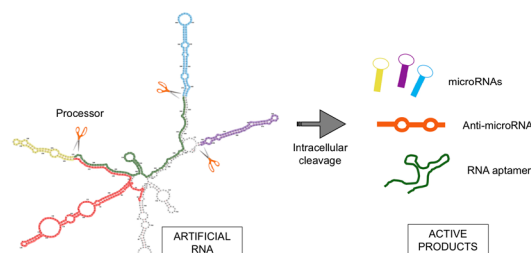


Figure 1. Structure of the artificial RNA and resulting bioactive products

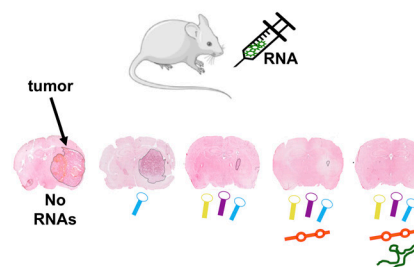


Figure 2. In vivo antitumor activity of progressively more composite RNAs



Regenerating T Cell Immunity

David Scadden, MD

Director, Center for Regenerative Medicine;
Co-Director, Harvard Stem Cell Institute, Director,
Hematologic Malignancies & Experimental
Hematology, MGH; Jordan Professor of
Medicine, HMS

dscadden@mg.harvard.edu

T cell immunity declines with age and acute thymic injury with marked health consequences. Infection, autoimmunity and cancer risk all increase as T cell immune function declines. It happens because the thymus, the body's T cell foundry, progressively decreases in function starting at puberty. Thus begins the gradual attrition of the T cell repertoire. There are currently no therapies to reverse it.

We took two complementary approaches to address this problem:

First, the thymus depends on the bone marrow to make T-competent cells. Specific cells in bone instruct blood stem cells to become T cell competent. These cells migrate to the thymus that completes the final T cell product. With Dr. David Mooney, we created a cell-free, off-the-shelf, subcutaneously injected cryogel that markedly enhances T competent progenitor cell and mature T cell generation. It increases T cell neogenesis, T cell receptor repertoire breadth and enhances functional immunity in animal models.

Second, we defined a stromal cell type in the thymus that governs cell entry and initiation of T cell differentiation. It does so through CCL19. Mesenchymal cells grown ex vivo bearing CLL19 injected into the thymus, restore atrophic thymus function, T cell neogenesis and T cell immunity. Each approach has been independently validated to overcome the acute T cell depletion and thymic injury state of bone marrow transplantation in animals.

These complementary but independent therapies require capital for further testing in non-human primates and development of clinical scale processes.

The envisioned clinical development path would be through two POC indications: 1. The setting of chronic age-related immune decline in patients who mount poor responses to clinically used vaccines for Influenza and/or Herpes zoster measuring TCR repertoire and response to neoantigen.

2. The acute immunodepleting process of allogeneic hematopoietic stem cell (bone marrow) transplantation measuring T cell recovery, TCR repertoire, CMV reactivity and infectious events at 100days.

Potential commercial opportunities include: 1. Cancer as complementary to immune checkpoint inhibitors; 2. Aged individuals with poor control of chronic viral infections; 3. Aged individuals with poor response to vaccines; 4. Patients undergoing allogeneic hematopoietic stem cell transplant.



Getting to the Heart of the Matter: Curing Genetic Cardiomyopathy

Christine Seidman, MD

Director, Cardiovascular Genetics Center, BWH; Smith
Professor of Medicine & Genetics, HMS

cseidman@genetics.med.harvard.edu

The ultimate opportunity presented by discovering the genetic basis of human disease is accurate prediction and disease prevention. To enable this achievement, genetic insights must enable the identification of at-risk individuals prior to end-stage disease manifestations and strategies that delay or prevent clinical expression. Genetic cardiomyopathies provide a paradigm for fulfilling these opportunities. Hypertrophic cardiomyopathy (HCM) is characterized by left ventricular hypertrophy, diastolic dysfunction with normal or enhanced systolic performance and a unique histopathology: myocyte hypertrophy, disarray and fibrosis. Dilated cardiomyopathy (DCM) exhibits enlarged ventricular volumes with depressed systolic performance and nonspecific histopathology. Both HCM and DCM are prevalent clinical conditions that increase risk for arrhythmias, sudden death, and heart failure. Today treatments for HCM and DCM focus on symptoms, but none prevent disease progression. Human molecular genetic studies demonstrated that these pathologies often result from dominant mutations in genes that encode protein components of the sarcomere, the contractile unit in striated muscles. These data combined with the emergence of molecular strategies to specifically modulate gene expression provide unparalleled opportunities to silence or correct mutant genes and to boost healthy gene expression in patients with genetic HCM and DCM. Many challenges remain, but the active and vital efforts of physicians, researchers, and patients are poised to ensure success.



Repurposed Tumor Cells as Killers and Immunomodulators for Cancer Therapy

Khalid Shah, PhD

Vice Chair, Neurosurgery Research, BWH; Director, Center for Stem Cell Therapeutics and Imaging, HMS
kshah@bwh.harvard.edu

Despite great leaps in the treatment of malignant neoplasms over the past decades, solid tumors remain the most challenging tumors to treat. Consequently, new therapeutic approaches are desperately needed, especially for solid tumors where standard therapy has failed and evidence-based options for salvage treatments are limited or lacking. We have developed an innovative and proprietary gene-edited and engineered tumor cell-based therapeutics for cancer, initially addressing significant unmet medical need in highly malignant-brain tumors, glioblastomas (GBM).

Tumor cells are known to exhibit a “self-homing” behavior, whereby cells released into the circulation can home back to the main tumor site. Utilizing resected tumor cells, we have created CRISPR/Cas9 edited bi-functional therapeutic tumor cells (ThTC) and shown that ThTCs simultaneously eliminate residual tumor cells and elicit an active immunity which prevents tumor recurrence in primary and recurrent GBMs as well as other primary and metastatic solid tumors. The incorporation of activatable dual kill switches into ThTC allows selective eradication of engineered tumor cells post-tumor treatment and ensures the safety of our approach. Our personalized strategy has a potential to significantly increase survival and minimize toxicity to normal tissues by reducing the undesirable side effects associated with other investigational drugs given to cancer patients.

Given the complexity of delivering drugs across blood brain barrier (BBB) and tumor resection/biopsy being part of standard of cure for GBM patients, our local ThTC treatment offers a ready availability for personalized cellular therapy for GBMs and serves as a potential cure for this devastating disease. Unlike other cell based therapies under development for treating GBMs, the ease of isolation, engineering of resected tumor cells and subsequent expansion of ThTC coupled with their off-the-shelf availability for recurrent disease should considerably reduce treatment costs in GBM patients.

We anticipate that our engineered tumor cell platform will have major contributions in finding a cure for GBM patients and is likely to define a new treatment paradigm for patients with other solid tumors.

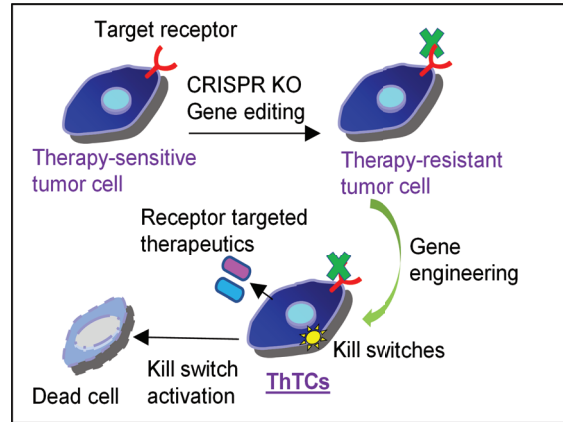


Figure 1. Creating gene-edited and engineered therapeutic-tumor cells (ThTC) from resected tumors.

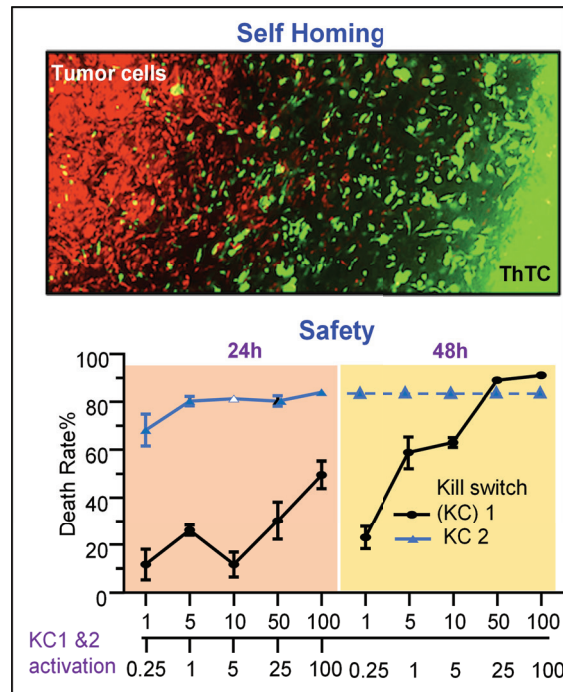


Figure 2. Self-homing and safety of patient derived gene edited and engineered ThTCs.



Control of AAV Pharmacology by Rational Capsid Design

Luk Vandenberghe, PhD

Grousbeck Family Chair, Gene Therapy, MEE;
Associate Professor, Ophthalmology, HMS

luk_vandenberghe@meei.harvard.edu

The adeno-associated viral vector (AAV) has emerged as the technology of choice for in vivo therapeutic gene transfer. Its desirable safety profile, its broad tissue targeting, and durable expression make it an attractive starting point to further optimize. However, the clinical adoption of novel, engineered AAV technology has been limited. A core constraint is the complexity of the AAV capsid, a highly conserved 60-mer particle structure with hundreds of protein-protein interactions required for stability and function. Current discovery approaches often violate this blueprint, leading to technologies with limited translational potential e.g. in terms of manufacturing. Rational design of AAV has been equally limited, as the structure-activity relationship of AAV remains poorly defined, particularly in a relevant in vivo context.

Here, we sought to develop an approach toward rational design of AAV for translationally relevant properties. Briefly, through computational, structural, and biological datasets, a library of variants is curated toward increased assembly competency and variation in putative functional groups. Once developed in the laboratory, these highly informative libraries are next screened in relevant model systems (e.g. non-human primate). Importantly, to address questions of ON versus OFF target transduction and biodistribution, pre-existing immunity, and manufacturability, a quantitative multiparametric screening approach, AAVSeq, was developed. AAVSeq relies on barcoding of the variant library and a cost-effective deep sequencing quantitative readout in parallel of all the properties needed for candidate selection.

In a proof-of-concept, we developed an AAV library based on maximum-likelihood ancestral sequence reconstruction and performed AAVSeq in vitro, as well as in mouse and monkey following an intravenous route of administration. These data illustrate the potency and potential of this approach for lead candidate selection, the mapping of key AAV structure-function attributes, and how to leverage these toward rational design of AAV vectors with improved properties to address translational limitations of the current generation of gene transfer vectors.

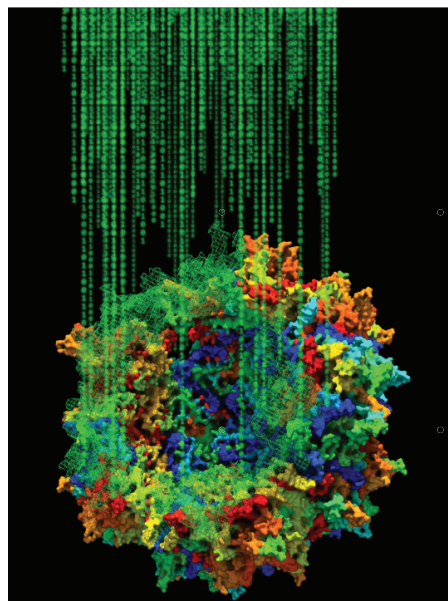


Figure 1.

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