HER2-targeted extracellular vesicles delivery of therapeutic mRNA for enzyme prodrug therapy

Wang JH1, Harada M1, Delcayre A2, Antes TJ3, Gupta A3, Kanada M4, Jeffrey S5, Pegram M6, Wilson C7, Grant G7, Contag C1,4, Matin AC1

1. Microbiology & Immunology, Stanford School of Medicine
2. ExoThera LLC
3. System Biosciences Inc.
4. Pediatrics, Stanford School of Medicine
5. Surgery, Stanford School of Medicine
6. Med/Oncology
7. Neurosurgery

Background: HER2 is overexpressed in aggressive breast cancers that have poor prognosis. We are using extracellular vesicles (EVs) the ‘body’s natural antigen delivery system’, for targeting our novel prodrug (CNOB/ChrR6) regimen specifically to HER2 positive cancer; the cytotoxic product of this regimen, MCHB, can be visualized noninvasively in living mice. EVs are thought to possess virus-like capacity for genetic material delivery without the safety issues of using viruses. Although our preferred approach is to deliver targeted EV-mediated therapeutic mRNA, which would bypass problems of using DNA for this purpose (e.g., ineffective transcription), we have also used DNA for gene delivery (Kanada, Ronald, Kim, Paulmurugan, Ge, Bachmann, Hardy, Gambhir, Matin, Contag; abstract submitted). Whether EVs can cross the blood brain barrier (BBB) was also investigated. The EV-based therapy holds the promise of personalized medicine using the vesicles derived from a patient’s own dendritic cells (DCs); EVs from these cells have the further advantage of harboring functional MHC-peptide complexes and various immunity-stimulating factors.

Aims: To use HER2-targeting EVs for specific and effective delivery of prodrug therapy to HER2 cancer.

Methodology: Using exosome-display technology employing p6mLSC1C2 plasmid, we have described before, we constructed a chimeric protein, anti-HER2 (ML39) scFv conjugated to the lactadherin C1C2 domain. After 4-day incubation of transiently transfected HEK293 cells, EVs were harvested (conditioned medium; ultracentrifugation), and pure chimeric protein was isolated using His-tag columns. Naïve HEK293 or DC EVs were reconstituted with the chimeric protein. ELISA quantified the specificity of the resulting HER2 targeting-EVs. For cell binding assays, CFSE-labeled targeted EVs were incubated with BT474 (strongly HER2+ve) or MCF7 cells (little HER2 expression), and visualized by fluorescence microscopy. To determine mRNA transfer capability, the directed EVs were loaded with ChrR6 mRNA by Xport-based technology, and incubated with BT474 cells followed by CNOB treatment. MCHB production was quantified by fluorescence at 584/612nm. DNA was introduced into the EVs using minicircle (MC) transfected cells. The cranial window model was used to visualize the mouse brain vasculature.

Results: Transfected HEK293 cells produced EVs expressing the anti-HER2 scFv/C1C2 chimeric protein. DCs lack lactadherin and generate naïve EVs. These were incubated with the chimeric protein. The resulting directed EVs showed 10 times greater targeting capacity than EVs from the transfected HEK293 cells (ELISA). They also displayed greater targeting activity to BT474 than to MCF7 cells (fluorescence microscopy). Directed, loaded EVs specifically conferred transcription-independent capacity on BT474 cells to activate CNOB (generation of MCHB fluorescence) showing successful delivery
of translationally-competent ChrR6 mRNA. Use of EVs from MC-transfected 4T1 cells showed improved EV-mediated DNA delivery and expression. EVs had a short circulation time, did not cross the normal BBB, but appeared capable of crossing the tumor vasculature.

**Conclusions:** We have successfully engineered EVs capable of high levels of HER2 binding capacity and functional delivery of therapeutic nucleic acids including mRNA. The directed EVs are currently being tested in mice with implanted orthotopic BT474 tumors as well as in xenograft models with patient-derived HER2+ve cancer cells in mice.