## Meeting Review:

*Cambridge Healthtech Institute's 2nd Annual*

**Oncolytic Virus Immunotherapy**

**Commercializing the Exciting Potential of Oncolytic Virotherapy**

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# Latest Developments in Oncolytic Virus Immunotherapy

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**Abstract**:

The Cambridge Healthtech Institute's 5th International Annual **Immuno-Oncology Summit** in Boston, Massachusetts brought together over 100 of the well-known players in oncolytic virology for their **Oncolytic Virus Immunotherapy** conference. The theme of this year's talks seemed to be a return to form. Almost all the speakers acknowledged the immense benefit combining OVs with checkpoint inhibitors brings and with that knowledge sought to improve the vector itself. The panel of speakers this year presented data on the right transgenes to arm OVs and systems biology approaches to discover the best viral backbones to engineer into vectors. Here we summarize the meeting's keynote talks, thematic principles running through the summit, and current developments in the OV field.

**Introduction**:

As with all good conferences, the keynote talk sets the unifying or dividing theme for the rest of the presenters to embellish or dispute. This year the theme was to build a more cytolytic virus and design viruses to better home to their cancer targets. John Bell, PhD, gave the keynote lecture, philosophizing to know how to design an OV, one must know the cancer they seek to cure. He focused on how transforming mutations, such as TP53 dominant negative mutants and gain of function VEGFR mutants, are known to drive tumorigenesis but also drive viral replication. Constitutive growth factor signaling represses anti-viral defenses allowing attenuated vectors to thrive in a cancer environment. However, researchers must be cautious as the growth factor

signaling from cancer cells also allows untransformed cells associated with the cancer to become permissive to vector mediated killing (1). The same pathways used to drive tumorigenesis are also used in normal wound healing which many OVs do not distinguish between, as observed during PexaVec infection of normal endothelial cells within tumors. Luckily these off target infections are centered around the tumor which opens the opportunity for designing new vectors to kill tumor associated support cells. To conclude Dr. Bell addressed the elephant in the room: Since the checkpoint inhibitors seem to work well with most OVs, what's left to do? Researchers and companies seem to have two choices: design more peptide and transcriptionally targeted viruses that inflict more damage to specific tumor tissues, or arm their vectors to the teeth, the more cytotoxic and immune-stimulatory the better.

**Closing the frontier: Better ways to target OVs into a tumor.**

A longstanding problem with viral vector therapy is how to maximize the amount of virus that gets into and stays within the tumor. This debate has many sides and proffered solutions. Direct injection of virus into the tumor or systematic delivery is the question every company taking a vector to trial wants to know, and often the answer varies based on the virus, cancer, or both. Both deliveries require vectors designed to specifically target tumor cells. To this end many novel ways of getting or keeping virus into a tumor were presented.

Transcriptionally targeting a virus requires a tumor cell specific expression pattern to drive replication of the virus. The γ34.5 genes in HSV1 are often removed in vectors designed for CNS cancers because of the associated neuropathology that their protein products cause. To get around this Antonio Chiocca's group designed the rQnestin34.5 oHSV with deletions of the endogenous γ34.5 genes and an insertion of an engineered nestin promoter driven γ34.5 into the ICP6 gene (2). Hiroshi Nakashima, PhD, of the Chiocca lab, presented his work demonstrating a safer way to target cancers was to replace γ34.5 with the partially homologous mammalian gene GADD34. This alteration remarkably improved the safety over the parental virus during intracerebral injection into mouse brains. This new virus, NG34, still reduces phosphorylation of eIF2α and increases the survival of mice harboring orthotopic glioblastoma tumors. NG34's efficacy in relation to the immune system was demonstrated using a fascinating model whereby the LCMV virus is used to create an exhausted T cell state prior to OV injection. This model mimics the T cell exhaustion observed in human patients' glioblastoma (GBM), and should be adapted for OV research due to the immense pressure researchers put on using viral vectors in conjunction with checkpoint inhibitors. Paola Grandi, PhD, co-founder of Oncorous provided another illuminating talk on transcriptionally targeting glioblastoma using an oHSV. The ONCR001 and ONCR002 viruses are targeted to GBM tumors via modifications to their gB surface proteins as well as a miRNA-124 targeting sequence in the critical HSV1 ICP4 gene to prevent replication in normal cells even if off target infection occurs. These viruses still possess the γ34.5 genes making them potent in destroying tumor cells while also reducing the IDO and suppressive nature of MDSCs. Dr. Grandi also boasted the discovery and creation of several viruses controlled by other miRNAs found in lung tumors, previously not utilized in OV therapy.

Adenovirus (Ad) vector enthusiasts, David Curiel, MD/PhD, and Clodaugh O'Shea, PhD, demonstrated insightful ways to target their vectors. Dr. Curiel focused on ways to get CRAd vectors out of the liver after systemic delivery. To do this a series of changes to the knob and fiber protein were made by Curiel's group to prevent liver accumulation. The H3 hexon was swapped to prevent serum factor X binding, and a RGD4 insertion was made into the fiber knob to detarget CAR. To follow up, fibritin, the whisker fiber protein from T4 bacteriophage, was swapped with the Ad fiber which allowed small chain antibody (scFv) attachments to the knob. These fiber-fibritin chimeras selectively infect cells expressing the scFv's target. Clodaugh O'Shea's group went steps further in her presentation, by demonstrating that the entire Ad genome is capable of modularly swapping in and out different strains subunits. Using a novel engineering approach her group can create potentially millions of chimeric Ad vectors to target tissues the virus has naturally evolved an affinity for. Utilizing this system, Dr. O'Shea's group engineered an Ad vector with a modified fiber which binds rapamycin, which can in turn be linked to a camelid antibody targeting receptors enriched on tumor cells, in this case EGFR. This allows a quick on/off targeting switch for her Ad vectors which is important due to the poor specificity of anti-adenovirus drugs available. In addition she presented findings on several modular Ad vectors in several tumor models to illustrate how much potential this creation system has for OV therapy.

The last talks focused on targeting OVs to tumors using novel methods to ferry virus to the tumor. Sari Pesonen of Valo Therapeutics presented a way of coating CRAd virions with immunogenic cancer peptides. Upon injection, the CRAd infects normally but upon infection provides a batch of cancer peptides to be presented on MHCI complexes along with co-stimulatory molecules due to the viral infection. This way the virus acts as an immune-stimulatory boost to retarget the immune system to cancer peptide. More directly, Dr. Aboody's graduate student Jennifer Batalla presented findings on loading neural stem cells with CRAd vectors. The carrier cells shuttle virus to cancer niches via chemokine gradients before being lysed by the virus and infecting nearby tumor cells. While preliminary, Batalla's data shows a trend towards synergy between CRAd loaded NSCs and Cisplatin.

**Armed and Dangerous: The dreadnaughts of armed vectors.**

'Bigger is better' comes to mind when examining the panel of armed OVs presented at this year's conference. While vectors of the past like TVEC/IMLYGIC and Toca-511 sport one transgene for tumor destruction, many of the vectors presented this year were armed with multiple factors. The ability to arm vectors with multiple transgenes speaks volumes to the advancement in vector engineering techniques as well as the ethos of pushing the boundaries of the familiar which lies at the heart of viral vector therapy.

The large size of herpes viruses makes them suitable vectors for engineering multiple arming vectors. To this end Rob Coffin, PhD, co-founded Replimune to improve oHSV vectors. He presented the RIP1-3 series of oHSVs. Replimune conducted a screen of several HSV1 strains to isolate one capable of growing on multiple cancer cell lines. HSV1 RH18A was chosen not based on its cytolytic effect on select lines, but its general cytolytic effect on all lines tested. To create the RIP vectors the γ34.5 genes and ICP47 were deleted for safety and immediate early expression

of Us11, respectively. In addition, a truncated gibbon ape leukemia virus GP (GALVP) protein was inserted. GALVP expressing RIP viruses spread better and provided a synergistic survival benefit with checkpoint inhibitors at low viral doses. Each RIP is armed with the previous RIPs transgenes as well as a new one. RIP1 expresses GM-CSF, RIP2 expresses a secreted CTLA4 antibody, and RIP3 expresses an additional immune stimulatory ligand which was not disclosed. Replimune aims to conduct clinical phase I and II trials in 2018 and 2019 with these viruses. Matthew Mulvey, PhD and CEO of Benevir offered his take on the immune system's unwanted effects on OV therapy. While OVs elicit an immune response, which helps kill tumor cells, Dr. Mulvey reminded the audience that its main target is the viral vector. To mitigate the immune systems unwanted viral targeting while keeping its anti-tumor benefits Benevir has developed T-Stealth™. T-Stealth has the same deletions and transgenes as TVEC/IMLYGIC, but expresses the bovine herpes virus $U_L$49.5 which inhibits the transporter associated with antigen processing (TAP) channel presentation of peptides on MHCI (3). Unlike HSV1 ICP47, the BHV $U_L$49.5 can inhibit mouse TAP. This allows Benevir to test the cloaking ability of T-Stealth in murine models, which the ICP47 deleted TVEC and other oHSVs are unable to accurately test. Supporting his theory, Steven Thorne's studies demonstrated checkpoint inhibitors can completely ablate vector replication and therapeutic effect when given too early (4). Embellishing on this study, Benevir testing showed T-Stealth provided better survival benefits, spread throughout tumors, and elicited a greater variety of TCRs in infected mice. In addition, Dr. Mulvey discussed the importance of incorporating vaccinated mice in future efficacy studies to model the high prevalence of patients that have pre-exiting memory responses to HSV1.

CSOs Steven Thorne of Western Oncolytics, and Brian Champion of PsiOxus, both showed the audience vectors armed with multiple immune-stimulatory ligands and effectors. Dr. Thorne began by describing WO-12 which is a TK-, C12L deleted vaccinia vector expressing IL-18. WO-12 is deglycosylated before injection to evade host antibody responses before infecting cancer cells. Deglycosylating vaccinia reduces TLR2 activation upon infection and shifts the innate immune response to a necroptotic TLR3 mediated cell death. To improve this shift, WO-12 was enhanced by expressing TIR-domain-containing adapter-inducing interferon-β (TRIF), which is a downstream effector of TLR3. WO-12 delays growth of tumors and causes a robust T cell mediated response to tumors. Piling more benefits on top, Thorne added another transgene, HGPD, which inhibits PGE2. In addition to the other transgenes, HGPD expression reduces the levels of MDSCs which inversely correlated with survival benefit in tumor models. In line with more is better, Dr. Champion presented the large number of PsiOxus Enadenotucirev (EnAd) vector modifications. The NGEnAd vectors are built from the EnAd backbone but express transgenes like FLT3L, IFNα, and M1Pα/$CCL_3$ (NG345), which promote a highly inflammatory immune response. NG347 expresses CD80, IFNα, and M1Pα/$CCL_3$, which also increases an immune response to virus and tumor. The NG601-611 vectors all express Bi-specific T-cell engagers (BiTEs) which target CD3 on T cells and a tumor antigen. PsiOxus' developed a humanized CD34 mouse model to test colon cancer where NG606 and NG611 virus expressed TAM or EPCAM targeted BiTEs which improved T cell recruitment and co-culture killing in vitro.

**Clinical Trials**

A dedicated session that included preclinical and clinical phases of oncolytic virus development was chaired and moderated by Dr. Fares Nigim from Massachusetts General Hospital – HMS and Yale NH/BP Hospital. Speakers were from both industry and academia. Dr. Naomi de Silva, associate director preclinical Science at Sillajen Biotherapeutics, Inc. elegantly presented her very recent clinical work and future plans on PexaVec. PexaVec is an oncolytic vaccinia virus that was shown to have a significant effect in increasing tumor cytotoxic T cell infiltration and disrupting the tumor vasculature by targeting tumor-associated endothelial cells when combined with checkpoint inhibitors (i.e., PD-1 inhibitor). Recent phase I/II trial that included patients with renal cell carcinoma who received intratumoral injection of PexaVirus showed complete regression of the tumor at 8 months after virus injection. A second phase Ib dose-escalation study for metastatic and unresectable renal cell carcinoma is being initiated. Moreover, PexaVirus is thought to have a significant effect on tumors that don't respond well to checkpoint inhibitors, therefore the company is designing a phase I/II trial in patients with advanced colorectal carcinoma with PexaVirus in combination with CTL4 and PD-1 inhibitors. Relevant results should be coming out soon.

Dr. Caroline Breitbach presented her work on the Maraba MG1 Phase I/II expressing MAGEA3 tumor peptide trial. Patients with untreatable NSCLC are injected with  and Ad/MAGEA3 to prime natural immunity before being administered with MG1-MAGEA3 as a single IM dose. Another MG1/MAGEA3 boost is administered IV on Day 15 and Day 18. Nanostring of patient samples shows up-regulation of immune stimulatory genes, and the trial is ongoing.

A very intriguing and interesting concept behind the use of flu virus as an oncolytic virus against solid cancers was presented by Dr. Michael Bergman, from the University of Vienna and co-founder, CMO at Vacthera. Vacthera presented its oncolytic flu virus armed with IL15 (into NS1 partial deletion) and expresses ESAT-6 transgene. ESAT-6 expressing NS1 deletion virus was shown to induce less TNF and therefore seems to have negative effect on the anti-virus innate immune system. Moreover, Dr. Bergman showed conditional growth of the virus in tumor cells. The company has developed a proprietary purification platform to reach high virus titers and a phase - I clinical trial in colorectal metastatic cancer is being planned with the aim to test the effect of oncolytic flu virus with checkpoint inhibitors.

**Conclusion:**

Oncolytic virus vectors are quickly becoming palatable to the larger medical community and private enterprise thanks to the breakthroughs of immunotherapy for cancer treatment. As more and more data is acquired from phase I and II studies the breakout of oncolytic virotherapy may emerge as the next great achievement in humankind’s battle against cancer. Hopefully the lessons from past trials and failures will illuminate us towards better vectors that can shape the future of modern medicine.

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For more information about the 2018 Oncolytic Virus Immunotherapy meeting please visit www.immuno-oncologysummit.com or email dbarry@healthtech.com