Development and testing of next generation oncolytic vaccinia viruses.

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Principal supervisor's CRUK CC theme:

- Cancer Immunology Programme
- Cell and Molecular Biology Programme

Department for student registration: Department of Pathology
Department or institute where research will take place: Department of Pathology

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Postgraduate scheme: **MRes + PhD (1 + 3-year non-clinical applicants only)**

MRes project outline:

Oncolytic viruses (OVs) have the potential are part of the therapeutic arsenal against cancer. Only one OV, however, is FDA approved for treatment of late-stage melanoma. Other OV trials have proven less successful, in part due to the lack of mechanistic understanding of how they clear tumours. Recent advances in understanding of tumour immunology allow us to more rationally design OVs to drive appropriate T cell responses that can more effectively drive tumour regression. Vaccinia virus (VACV) is a large double-stranded DNA virus that has excellent potential as an OV. VACV was used as the vaccine to eradicate smallpox and hence has an excellent safety profile and capacity to drive memory immunity. It can be engineered to replicate specifically in tumour cells and can accept large inserts into its genome as payloads. In this project you will learn how to engineer VACV to make new candidate OVs based on their ability to activate specific immune signalling pathways in tumours. You will knockout specific genes from the virus based on our knowledge of the function of those individual genes in order to improve the ability of VACV to drive specific inflammatory signalling pathways in the tumour. Having made new OVs, you will assess their capacity to drive the expected anti-tumour immune responses in cellular and organoid models. The specific aims of the project are therefore, i) to design, make and quality control new VACV-based OVs and ii) to assess their ability to generate specific inflammatory signals in normal and tumour cell and organoid cultures.

MRes experimental plan:

New OV's will be generated by molecular cloning of gene-knockout recombination-plasmids followed by transient dominant selection methods to generate recombinant VACVs. PCR and sequencing methods will be used for quality control of newly generated OVs. Plaque assay titrations will be used to quantify viruses. Cell culture, organoid culture, virus infection immunoblotting and ELISA will be used for analysis of OVs in cell lines.

PhD project outline:

Oncolytic virotherapy, despite being clinically relevant, is currently limited by the ability of OVs to effectively drive anti-tumour immune responses. In this project you will develop and test new OVs based on VACV that specifically activate T cell immunity from within the tumour microenvironment by driving the activation of dendritic cell populations and epitope spreading to present tumour-specific antigens.

We know that the efficacy of oncolytic virotherapy is partly dependent on the ability of the virus to trigger anti-tumour immune responses. For example DNA-based oncolytic viruses, such as vaccinia virus, can trigger pattern recognition receptors that drive interferon and cytokine production via the STING/IRF3 and NF-kB pathways. These viruses, however, produce multiple countermeasures that block these pathways. We know from infectious disease vaccination development that deletion of these viral inhibitors can trigger stronger innate, cellular and adaptive immune responses. Using this knowledge we have rationally deleted multiple genes from vaccinia virus to generate new oncolytic viruses that trigger specific host innate pathways.

Our range of novel OVs have the capacity to drive specific inflammatory signalling pathways from within the tumour and now these are being modified to target specific cancers and to express immunostimulatory payloads. In this project you will design, create and test further OVs based on our current range of candidate target OVs by tagging the surface of the virus with tumour-targetting antibodies and by expressing T cell checkpoint inhibitors from the virus. You will test the replication, tumour lysis and innate immune stimulation capacity in vitro in tumour lines and organoid models and assess efficacy in tumour models. Finally you will assess the synergy of these viruses with DNA damaging chemotherapeutics and irradiation therapy.

The objectives of the project are therefore:
1- Deletion of genes from vaccinia virus that block specific innate immune signalling pathways
2- Engineer into these viruses to express specific immunostimulatory payloads.
3- Define the innate sensing pathways triggered by these viruses in tumour cells with known cancer driver mutations
4- Test the ability of these viruses to replicate and kill tumour cells and human organoids
6- Assess the synergy of novel OVs with DNA damaging agents

PhD experimental plan:

New OV's will be generated by molecular cloning of gene-knockout recombination-plasmids followed by transient dominant selection methods to generate recombinant VACVs. PCR and sequencing methods will be used for quality control of newly generated OVs. Plaque assay titrations will be used to quantify viruses. Cell culture, virus infection immunoblotting and ELISA will be used for analysis of OVs in cell lines. Specific combinations of genes eg B2/E5/N2 will be deleted to specifically unblock (and therefore enhance) intratumoural pattern recognition receptor signalling and immunogenic cell death during treatment. Following manufacture, new viruses will be quality controlled by PCR and sequencing to ensure appropriate deletion. These newly generated VACVs will be used to infect primary fibroblasts, tumour cell lines and human organoids in culture, and intracellular PRR signalling activation will be quantified by immunoblotting and ELISAs. Syngeneic tumour models will be used to assess efficacy of the OV's in vivo.

Main techniques:

Virus engineering and quality control, innate immune signalling assays, tumour models, immunephenotyping, organoid culture.

Key references:

Oncolytic Poxviruses Winnie M. Chan and Grant McFadden Annual Review of Virology 2014 1:1, 191-214

Activation of interferon regulatory factor 3 by replication-competent vaccinia viruses improves antitumor efficacy mediated by T cell responses, Stephanie Riederer, Robert Fux, Michael H. Lehmann, Asisa Volz, Gerd Sutter, Juan J. Rojas, Molecular Therapy - Oncolytics ,2021, Pages 399-409,

Vaccinia virus immune evasion: mechanisms, virulence and immunogenicity
Geoffrey L Smith 1, Camilla T O Benfield 1, Carlos Maluquer de Motes 1, Michela Mazzon 1, Stuart W J Ember 1, Brian J Ferguson 1, Rebecca P Sumner J Gen Virol 2013 Nov;94(Pt 11):2367-2392

Vaccinia virus protein N2 is a nuclear IRF3 inhibitor that promotes virulence Ferguson, B. J., Benfield, C., Ren, H., Lee, V. H., Frazer, G. L., Strnadova, P., Sumner, R. P., & Smith, G. L. (2013).. The Journal of general virology, 94(Pt 9), 2070–2081

A mechanism for the inhibition of DNA-PK mediated DNA sensing by a virus. NE Peters, BJ Ferguson, M Mazzon, AS Fahy, E Krystofinska, R Arribas-Bosacoma, LH Pearl, H Ren, GL Smith PloS Pathogens (2013) 9(10): e1003649

Increased attenuation but decreased immunogenicity by deletion of multiple vaccinia virus immunomodulators RP Sumner, H Ren, BJ Ferguson and GL Smith, (2016) Vaccine 34 (40), 4827-4834