CLINICAL VIROTHERAPY AND IMMUNE MODULATION – BENCH TO BEDSIDE AND BACK AGAIN

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BACKGROUND

• Oncolytic viruses (OV) were initially developed as direct cytotoxic agents.
• Can be naturally occurring (reovirus) or genetically modified (HSV, VV)
• We, and others, have shown that they can also act by activating an anti-tumour immune response.
• HSV.GMCSF (T-Vec) completed Phase III as intratumoural therapy for melanoma – trial met its primary endpoint of durable response rate.
• Little known about the biological effects of OV in humans.
BACKGROUND

- Reovirus is a dsRNA virus which targets cells with activation of the ras pathway (?).
- Almost everyone exposed to reovirus in childhood and so NAB positive.
- Previous lab work: In mouse and human pre-clinical systems reovirus is effective by direct and immune-mediated effects, alone or in combination with chemotherapy or radiotherapy.
- Reolysin has been through Phase I/II trials.
- I.t. and i.v. delivery, single agent and in combination with chemotherapy or radiotherapy.
- Phase III trial in head and neck cancer in platinum-resistant disease: carbo/taxol +/- reovirus (REO 18).
REO 13 TRIAL – A BIOLOGICAL ENDPOINT STUDY

- Patients with colorectal cancer metastatic to the liver, planned for radical surgery.
- One cycle of single agent iv reovirus (5 daily treatments), 1 – 5 weeks before surgery.
- Endpoints were immune response and analysis of blood, tumour and normal liver.
- An experiment in a patient.

![Graphical representation of the trial timeline and blood collection points.](image-url)
All patients are positive for NAB at baseline
Peak response is generally at surgery timepoint
FATE OF REO AFTER IV DELIVERY

• After intravenous delivery in patients:
• Reovirus RNA is present in plasma and associated with PBMC, granulocytes and platelets (not red blood cells), but this is transient.
• Cell-associated, but not plasma, reovirus is ‘functional’ ie able to replicate and kill after hand-off to target cells.
• Cell-associated virus can potentially deliver virus to target tumour cells in patients.
CELL CARRIAGE BY GRANULOCYTES

Neat:
Amplified 10-1:
Amplified 10-2:

(AMP)

7 | 8 | 9 | 10 | - | + | - | +

7: 8: 9: 10: 10-1 10-2 10-3 10-4 10-5 10-6 UN

Reo:

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STAINING FOR REOVIRUS PROTEIN IN TUMOUR

9 out of 10 patients positive: 3 scored as weak, 6 scored strong

weak staining

strong staining

Tumour staining always stronger in tumour than surrounding stroma or normal liver
Hepatocyte staining: 5 patients negative, 5 patients faint staining
Consistent with some delivery to liver as indicated by transaminitis
In all 4 patients viral plaques could be retrieved from tumour but not normal liver.
CONCLUSIONS

• Reovirus is delivered preferentially to tumours after iv infusion despite the presence of NAB at baseline.
• Reovirus RNA appears only transiently in blood.
• ‘Functional’ virus is associated with cells; plasma virus is neutralised.
• Virus injection triggers early, innate immune response reflected by clinical side effects/rise in CRP, which clears virus before rise in NAB.
• Replication-competent virus can be selectively retrieved from tumour at the time of surgery.
• Pre-operative delivery safe, paving the way for neoadjuvant studies.
BACK TO THE LAB – HOW TO IMPROVE DELIVERY/ThERAPY?

• Boosting the number and/or activation state of virus carrier cells may enhance both delivery to tumour and immune-mediated reovirus therapy.

• Tested reovirus in mice in combination with GMCSF, GCSF, IL-2.

• Continued focus on systemic delivery, rather than ex vivo loading of carrier cells, which is clinically challenging and expensive.

• ‘In vivo loading of cell carriers for systemic delivery of reovirus’.
IN VIVO LOADING OF CELL CARRIERS FOR SYSTEMIC DELIVERY OF REOVIRUS

• Granulocytes/macrophages – GM-CSF.
• Granulocytes – G-CSF.
• Lymphocytes – IL-2.
IN VIVO LOADING OF CELLS CARRIERS IN COMBINATION WITH GM-CSF LEADS TO CURE OF S.C. TUMOURS EVEN IN PRE-IMMUNIZED MICE
THERAPY ASSOCIATED WITH A SINGLE CYCLE OF GM-CSF/REO IS SIGNIFICANTLY BETTER IN PRE-IMMUNE MICE (3 DAY ESTABLISHED S.C. TUMOUR)
GM-CSF/REO is also highly effective against well established tumour (pre-immune; 10D established S.C.).
IN VIVO LOADING OF CELL CARRIERS FOR SYSTEMIC DELIVERY OF REOVIRUS

• NAB-reovirus pre-bound complexes can be handed onto tumour cells from macrophages in vitro.

• GM-CSF activated splenocytes/lymph node cells kill target reovirus-resistant tumour cells in vitro in the presence of NAB-reovirus complexes – dependent on NK cells and macrophages.
IN VIVO THERAPY WITH REO/GMCSF IN PRE-IMMUNE MICE IS DEPENDENT ON NK CELLS AND MONOCYTES, BUT NOT T CELLS OR NEUTROPHILS

Antibody depletion of:
Ly-6C+ve cells - expressed on neutrophils and monocytes
Ly-6G+ve - expressed on neutrophils
IN VIVO THERAPY ALSO SEEN IN S.C. TC2 PROSTATE TUMOURS IN PRE-IMMUNE MICE

The graph shows the percent survival over days for different groups:
- Control
- reo
- GM-CSF
- GM-CSF + reo

The y-axis represents percent survival, and the x-axis represents days.
SOME EFFECT ALSO SEEN WITH IL-2, BUT NOT WITH G-CSF
GM-CSF CONDITIONING FACILITATES EFFECTIVE SYSTEMIC THERAPY WITH REOVIRUS

Activated mono/macs:
- FcR +ve
- Tumour trafficking/viral delivery

TUMOUR CELL

GM-CSF activated Mono/mac Tumour trafficking
TUMOUR

Virus release
- Replication
- Oncolysis
- Anti Viral Immune Response

Anti-Viral Innate Immune Response; NK Cells; Others

GM-CSF CONDITIONING FACILITATES DIRECT AND INNATE IMMUNE-MEDIATED KILLING

GM-CSF activated Mono/macs
- Fc Receptor +ve
- Tumour trafficking

TUMOUR

TNF-α ? others

NK
NEXT TRIAL

- Biological endpoint study.

- Reovirus +/- GMCSF prior to planned resection of advanced melanoma.

- Patients will be pre-immunised against the virus.
BRAIN TUMOURS

• Both primary and secondary are major unmet clinical need.

• Most oncolytic virotherapy given by direct injection.

• But can systemic oncolytic viruses cross blood brain barrier?

• Far easier for widespread clinical application to multifocal/infiltrative disease.
PRE-CLINICAL DATA

- Reovirus can enter CNS via haematogenous spread in mice.
- Confirmed reovirus can cross BBB in mice and selectively infect implanted melanoma (even in pre-immune mice).
- Early suggestion that access may be enhanced by radiotherapy.
THERAPY WITH GM-CSF/REO IN PRE-IMMUNE MICE IN MELANOMA REOVIRUS-SENSITIVE BRAIN METASTASIS MODEL

5d Established Intra-cranial B16 Tumours Treated with 3 Cycles of GM-CSF/REO
THERAPY WITH GM-CSF/REO IN PRE-IMMUNE MICE IN MELANOMA REOVIRUS-RESISTANT BRAIN METASTASIS MODEL

Established Intra-cranial B16.OVA Tumours Treated with GM-CSF/REO
5d Established intra-cranial GL261 Tumours Treated with 3 Cycles of GM-CSF/REO

Currently working on combination with targeted radiotherapy/chemotherapy
BACK TO THE CLINIC - REO13 BRAIN STUDY

- Phase 1b translational trial.
- Preoperative intravenous reovirus.
- Recurrent high grade primary or metastatic brain tumours.
- Can intravenous virus access tumours in the brain in patients?
- Similar design to REO13, administering a single iv infusion of reovirus, prior to resection.
- 4 patients treated, 3 analysed - tumour positive for reovirus in all 3 analysed.
Patient 1 - GBM

No primary antibody (protein)          Primary & brown secondary (protein)

Reoviral RNA – blue is positive

Primary & brown secondary (protein)
Patient 2 - colorectal adenocarcinoma

No primary antibody (protein)

Reoviral RNA (blue is +); viral protein is red, cells expressing viral RNA and protein are yellow

Primary & brown secondary (protein)

Primary & red secondary (protein)
Patient 3 – grade 3 oligodendroglioma; less positive

No primary antibody (protein)  Primary & brown secondary (protein)

Reoviral RNA – blue is positive  Primary & red secondary (protein)
### Trial: Phase I/randomised Phase II Trial of Reovirus/GM-CSF in combination with chemoradiation following surgery for high grade glioma

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SUMMARY

• Systemic delivery is feasible in patients in the face of NAB.
• Intravenous oncolytic viruses can access tumours in the brain in patients.
• Iterative lab to clinic research is most likely to deliver patient benefit, but does not substitute for therapeutic clinical trials.
• Parallel (not sequential) lab and clinical studies help progress.
• Rational combinations with current or deliverable immunomodulatory therapies (as well as standard chemo- and radiotherapy), in the laboratory and clinic are likely to enhance the benefit of oncolytic viruses.
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