Common Cold Virus as a Cancer-Killing Agent

Victoria Roulstone,
BSc (Hons) Biochemistry,
Higher Scientific Officer,
The Institute of Cancer Research.
Chester Beatty Laboratories,
Fulham Road, London, UK.

Correspondence:
E: Victoria.Roulstone@icr.ac.uk

Incidence rates of cancer have increased by almost a third since the mid-1970s and, while radiotherapy and chemotherapy can be curative, many types of cancers do not respond. (http://www.cancerresearchuk.org/cancer-info/cancerstats/%20keyfacts/Allcancerscombined/). There is therefore a continuing need for new, safe and effective therapies, with minimal toxicity to normal tissue. Interest in viral therapy to treat cancer began at least 100 years ago with reports of tumour remission following natural viral infection or vaccination [1]. Reovirus is an unmodified virus found ubiquitously in the environment, with most adults having been exposed in their lifetime [2]. Reovirus replicates in the respiratory and gastrointestinal tracts of humans, and symptoms of infection are mild [3]. In the 1970s, it was discovered that normal cells transformed into cancer-like cells were more sensitive to infection by reovirus, sparking interest in the field of oncolytic viral therapy [4]. This increase in sensitivity was connected to overexpression of aberrant signaling pathways often mutated in cancers [5]. This overexpression allows reovirus to replicate effectively in cancerous cells, whereas normal cells respond to infection by shutting down viral replication [6].

Reovirus type 3 (Dearing) (RT3D) in the clinic

RT3D was first tested in the clinic in 2001 for its safety as a single agent, injected directly into the tumours of patients with advanced cancers for which all other standard therapies were unsuitable. Treatment was well tolerated, with headache and flu-like symptoms, but responses were modest [7-9]. With many cancers inaccessible for direct injection, it was tested by systemic intravenous administration in clinical trials. Patients received one dose/day for five consecutive days, every four weeks. Again, side-effects were mild, and responses were limited to disease stability [10,11].

RT3D with radiotherapy in patients with advanced cancers

After trials testing RT3D as a single agent, subsequent strategies focused on combining RT3D with existing treatments that could potentially enhance its effects, such as radiotherapy. Pre-clinical studies showed that the combination of RT3D and radiation caused significant enhanced cytotoxicity in a panel of tumour cell lines, and correlated with an increase in apoptosis. Radiation did not affect viral replication, and reassuringly did not significantly reduce RT3D activity at clinically relevant doses [12]. The data supported a Phase-I trial of intra-tumoural reovirus with palliative radiotherapy in patients with advanced cancers. The combination treatment was well tolerated, and of the 23 patients treated, 14 displayed stable disease or a partial response [13].

This cooperative cytotoxicity with existing standard treatments was investigated by combining RT3D with chemotherapeutic agents. Pre-clinical tests showed that reovirus synergized with various chemotherapies, leading to increased apoptosis [14-16]. This has been translated into the clinic in a number of trials.

Reovirus with chemotherapy in patients with advanced cancers

A Phase-I study of RT3D in combination with gemcitabine commenced in patients with advanced cancers whereby RT3D was administered intravenously every day for five days of a three week cycle [17]. Gemcitabine was given before the RT3D injections on day one and again on day eight. However, this treatment caused dose-limiting toxicity in the first two patients enrolled on the trial and the protocol was amended so that only one dose of RT3D was given on day one. Of 10 patients, disease remained stable in six of them. One patient had a minor response, and one patient a partial response.

A Phase-I study of RT3D in combination with docetaxel was much better tolerated, with no dose-limiting toxicity. RT3D was administered intravenously on every day for five days of a thee-week cycle, with docetaxel given before RT3D on day one [18]. Of the 24 patients receiving treatment, 16 were eligible for response assessment, of whom 14 (88%) had a complete response, partial response or stable disease.

RT3D has also been combined with two chemotherapeutic agents as a doublet therapy [19]. Combination of RT3D with both carboplatin and paclitaxel has shown promising potential in the clinic and Phase-III trials are underway. In the

Table 1. Clinical trials involving RT3D. Dose escalations, cohorts and numbers of patients enrolled in the study.

PHASE I STUDIES RT3D	Cohort	Reovirus dose (TCID50)	Treatment doses (Reovirus)	Chemo dose mg/m2	Radiation dose Gy	Number of Patients
Intravenous	1-8	Escalating ($1x10^8$ - $3x10^{10}$)	Escalating 1,3,5			33
Intratumoural + Radiation Phase 1a	1-3	Escalating ($1x10^8$ - $1x10^{10}$)	2		20 (x5, days 1-5)	11
Intratumoural + Radiation Phase 1b	4-6	$1x10^{10}$	Escalating 2,4,6		36 (x12, days 1-16)	12
Intravenous + Gemcitabine	1-5	Escalating ($3x10^9$ - $3x10^{10}$)	5, 1*	1000		17
Intravenous + Docetaxel	3	Escalating ($3x10^9$ - $3x10^{10}$)	5	75		24
Intravenous + Carboplatin + Paclitaxel	3	Escalating ($3x10^9$ - $3x10^{10}$)	5	5 (C)/175 (P)		15
Intravenous + Cyclophosphamide	9	$3x10^{10}$	5	Escalating 25-1000		38

* Commenced trial with 5 doses but reduced to 1 after the first cohort.

Phase-I study, in keeping with previous trials, RT3D was administered every day for five days of a three-week cycle, with the chemotherapy doublet treatment given on day one before RT3D [20]. Treatment was well tolerated and responses were encouraging, particularly in patients with relapsed/metastatic head and neck cancer, for which all other available therapies had been heavily used and exhausted. The average overall survival was 8.9 months, with two patients showing clinically meaningful responses (resolution of cutaneous disease), six patients having a partial response, and one patient with a complete response (Figure 1). These results have paved the way for the randomized, two-arm, double-blind, multicenter, two-stage adaptive Phase-III study of carboplatin-paclitaxel, with or without RT3D, in patients with platin-refractory relapsed head and neck cancer. The results of this ongoing study are eagerly awaited (http://clinicaltrials.gov/ct2/show/NCT01166542).

Future Trials

Neutralising antibodies are a major barrier to effective tumour delivery in the oncolytic viral field, particularly with RT3D, where up to 100% of patients are pre-immune, blocking the virus path to the tumour [2]. Transient immunosuppression may facilitate viral delivery to distant tumours by reducing the level of antibodies that accumulated during treatment with RT3D. Pre-clinical data in a murine melanoma model indicate that cyclophosphamide is a potential immunosuppressive agent. Precise scheduling of cyclophosphamide in combination with reovirus resulted in high levels of intra-tumoural access and replication with only mild side-effects [21]. This has been translated to the clinic where the primary objective is to follow patient immune responses by careful and frequent monitoring of neutralising antibodies. An unmodulated antibody response will hinder repeated administration of RT3D, but ablation of antibody levels may lead to unwanted dissemination and toxicity. A Phase-I study is currently underway assessing the combination of RT3D with dose escalation of cyclophosphamide in patients with advanced malignancies.

Recent discoveries: cell carriage and delivery of RT3D

A recent window-of-opportunity study took place on patients due to undergo surgical removal of liver metastasis [22]. Prior to surgery, patients received five doses of RT3D intravenously over five days. In four patients, fresh tumour was excised during surgery along with adjacent normal liver tissue. Functional virus was isolated from all the tumours of these four patients, but it was not seen in the normal liver tissue. It is worth noting that 2/4 patients received fewer than the five doses of RT3D, raising the question of whether smaller doses are sufficient (or even advantageous) for therapy.

Secondary objectives of this study were to monitor humoral and cellular immune responses to RT3D following treatment. Interestingly, functional cytotoxic RT3D was found in patient peripheral blood mononuclear cells, granulocytes and platelets, despite the presence of functionally neutralising anti-reovirus antibodies before viral infusion. Hence, RT3D could be protected from neutralising antibodies after systemic administration by immune cell carriage, delivering RT3D to tumour. These findings will be important in future scheduling and treatment combination strategies to enhance immune evasion and effectively deliver RT3D to the tumour.

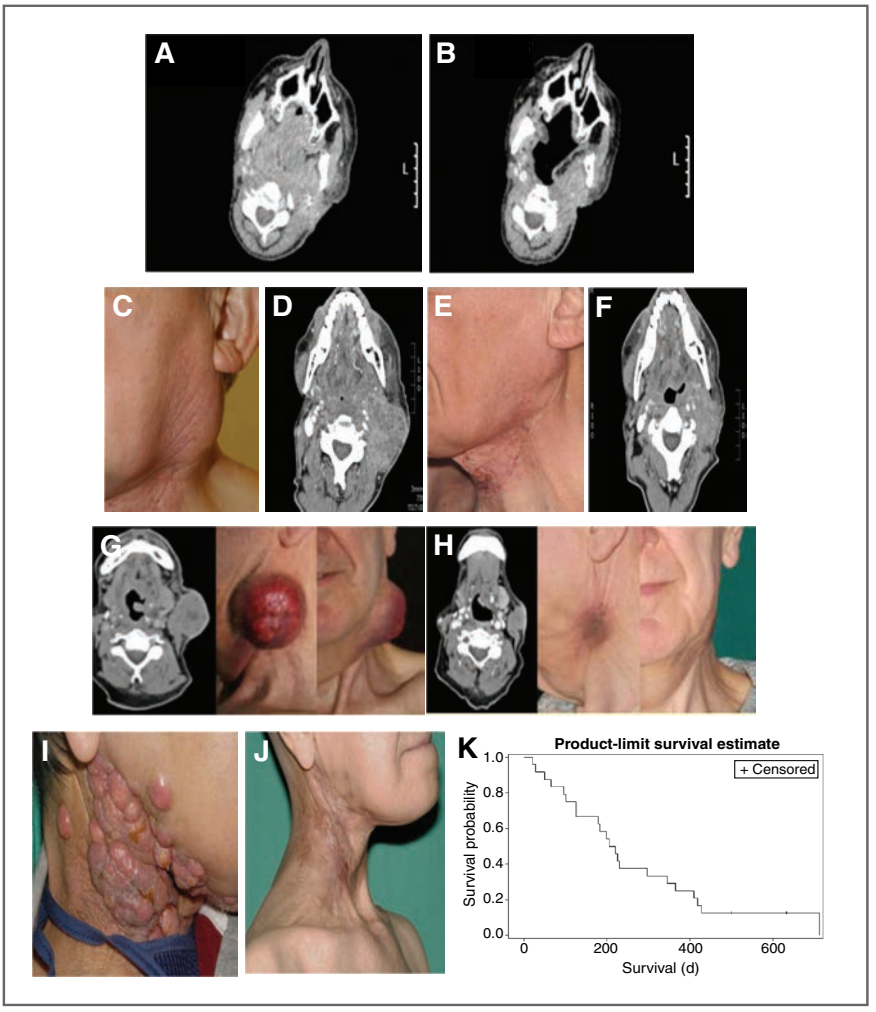

Figure 1: Treatment responses in patients in a phase I study of intravenous reovirus combined with platin/taxane doublet in patients with incurable or relapsed/metastatic head and neck cancer. Computed tomographic imaging before (A) and after three cycles of treatment with reovirus given with carboplatin and paclitaxel (B). This patient had oropharyngeal cancer (tonsil). The response was sustained through eight cycles of treatment, despite previous treatment with chemoradiation, palliative chemotherapy (cisplatin and 5-fluorouracil), and targeted therapy with an investigational monoclonal antibody. Before (C and D) and after three cycles of treatment (E and F). The patient had a supraglottic SCC and had been given prior treatment with chemoradiotherapy and two lines of palliative chemotherapy. Before (G) and after three cycles of treatment (H) This patient had a poorly differentiated SCC of the tongue who had been given prior treatment with surgery, radiotherapy and three lines of palliative chemotherapy – before (I) and after three cycles of treatment (J). The patient had a recurrent SCC of the hypopharynx, and displayed a major clinical response with treatment. Kaplan-Meier survival curves were plotted (K) for all patients with recurrent head and neck cancers; survival is shown in days, n = 24.
Originally published in Clin Cancer Res 2012;18:2080-9.

Conclusion

RT3D is an attractive oncolytic therapy, which is well tolerated as a single agent injected intra-tumourally or intravenously, or in combination with existing standard therapies. Whilst responses to RT3D treatment as a single agent are modest, recent encouraging observations in the clinic warrant further investigation as part of a dual or triple therapy causing cooperative cytotoxicity, in combination with both existing treatments and new novel therapies. ■

References

1. Nelson NJ. *Viruses and cancer.* J Natl Cancer Inst 1999;91(20):1709.
2. Minuk GY, Paul RW, Lee PW. *The prevalence of antibodies to reovirus type 3 in adults with idiopathic cholestatic liver disease.* J Med Virol 1985;16(1):55-60.
3. Rosen L, Evans HE, Spickard A. *Reovirus infections in human volunteers.* Am J Hyg 1963;77:29-37.
4. Coffey MC, Strong JE, Forsyth PA, Lee PW. *Reovirus therapy of tumors with activated Ras pathway.* Science 1998;282(5392):1332-4.
5. Strong JE, Coffey MC, Tang D, Sabinin P, Lee PW. *The molecular basis of viral oncolysis: usurpation of the Ras signaling pathway by reovirus.* EMBO J 1998;17(12):3351-62.
6. Mundschau LJ, Faller DV. *Endogenous inhibitors of the dsRNA-dependent eIF-2 alpha protein kinase PKR in normal and ras-transformed cells.* Biochimie 1994;76(8):792-800.
7. Don G Morris PAF, Alexander H Paterson, Kevin Fonseca, Lisa M Difrancesco, Brad G Thompson, Matthew C Coffey. *A phase I clinical trial evaluating intralesional Reolysin (reovirus) in histologically confirmed malignancies.* In: 2002 ASCO Annual Meeting; 2002: Proc Am Soc Clin Oncol; 2002.
8. Forsyth P, Roldan G, George D, et al. *A phase I trial of intratumoral administration of reovirus in patients with histologically confirmed recurrent malignant gliomas.* Mol Ther 2008;16(3):627-32.
9. Harrington KJ, Vile RG, Melcher A, Chester J, Pandha HS. *Clinical trials with oncolytic reovirus: moving beyond phase I into combinations with standard therapeutics.* Cytokine Growth Factor Rev 2010;21(2-3):91-8.
10. Vidal L, Pandha HS, Yap TA, et al. *A phase I study of intravenous oncolytic reovirus type 3 Dearing in patients with advanced cancer.* Clin Cancer Res 2008;14(21):7127-37.
11. Galanis E, Markovic SN, Suman VJ, et al. *Phase II Trial of Intravenous Administration of Reolysin((R)) (Reovirus Serotype-3-dearing Strain) in Patients with Metastatic Melanoma.* Mol Ther 2012;20(10):1998-2003.
12. Twigger K, Vidal L, White CL, et al. *Enhanced in vitro and in vivo cytotoxicity of combined reovirus and radiotherapy.* Clin Cancer Res 2008;14(3):912-23.
13. Harrington KJ, Karapanagiotou EM, Roulstone V, et al. *Two-stage phase I dose-escalation study of intratumoral reovirus type 3 dearing and palliative radiotherapy in patients with advanced cancers.* Clin Cancer Res 2010;16(11):3067-77.
14. Pandha HS, Heinemann L, Simpson GR, et al. *Synergistic effects of oncolytic reovirus and cisplatin chemotherapy in murine malignant melanoma.* Clin Cancer Res 2009;15(19):6158-66.
15. Sei S, Mussio JK, Yang QE, et al. *Synergistic antitumor activity of oncolytic reovirus and chemotherapeutic agents in non-small cell lung cancer cells.* Mol Cancer 2009;8:47.
16. Heinemann L, Simpson GR, Boxall A, et al. *Synergistic effects of oncolytic reovirus and docetaxel chemotherapy in prostate cancer.* BMC Cancer 2011;11:221.
17. Lolkema MP, Arkenau HT, Harrington K, et al. *A phase I study of the combination of intravenous reovirus type 3 Dearing and gemcitabine in patients with advanced cancer.* Clin Cancer Res 2011;17(3):581-8.
18. Comins C, Spicer J, Protheroe A, et al. *REO-10: A Phase I Study of Intravenous Reovirus and Docetaxel in Patients with Advanced Cancer.* Clinical Cancer Research 2010;16(22):5564-72.
19. Roulstone V, Twigger K, Zaidi S, et al. *Synergistic cytotoxicity of oncolytic reovirus in combination with cisplatin-paclitaxel doublet chemotherapy.* Gene Ther 2012.
20. Karapanagiotou EM, Roulstone V, Twigger K, et al. *Phase I/II trial of carboplatin and paclitaxel chemotherapy in combination with intravenous oncolytic reovirus in patients with advanced malignancies.* Clin Cancer Res 2012;18(7):2080-9.
21. Qiao J, Wang H, Kottke T, et al. *Cyclophosphamide facilitates antitumor efficacy against subcutaneous tumors following intravenous delivery of reovirus.* Clin Cancer Res 2008;14(1):259-69.
22. Adair RA, Roulstone V, Scott KJ, et al. *Cell carriage, delivery, and selective replication of an oncolytic virus in tumor in patients.* Sci Transl Med 2012;4(138):138ra77.