Development of a Trojan horse oncolytic virotherapy for treatment of myeloma.

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INTRODUCTION

Multiple myeloma remains an essentially incurable malignancy. In the vast majority of cases, tumour bulk may be substantially reduced by conventional and novel chemotherapies. However, almost invariably, disease re-accumulates and patients eventually succumb. Novel strategies to eliminate residual plasma cells are urgently needed.

Viral therapies for cancer have recently been developed and have shown promise in a number of models:

- Recently we have developed a novel Trojan horse virus system that shows superior promise in murine myeloma xenograft models. Most macrophages are hijacked and used as “Trojan Horses” as these cells naturally home to tumours, particularly to treatment resistant hypoxic regions. In previous studies, macrophages were loaded with an Adenovirus whose replication gene (E1A) was placed under the control of protease-specific promoter elements (NAP1/FF145) and replication plasmid that is activated under hypoxia (HRE-E1A). Following systemic administration, the macrophages homed to the tumour and release their virus cargo to infected and destroyed the surrounding tumours (Figure 1a)

- Adenovirus (Ad) is an engineered tumour-specific herpes simplex derived ON HSV1716 effectively kills tumour cell lines in vitro and in vivo when in low plaque-forming unit (PFU), HSV-1 1716 myeloma cells are treated with bortezomib 

- The percentage of macrophage infiltration between control tissue and tumour tissue was then assessed using ImageJ software.

METHODS

For the Trojan Horse system to work our myeloma models require macrophage infiltration of the tumour. To test this, NSG mice were implanted with 1 million human myeloma cells (U226). Tumours were left to develop for 21 days at which time mice were sacrificed and tissues taken for analysis. Sections of mouse tissue were stained with the macrophage marker F4/80 or for hypoxic HIF-1α. The percentage of macrophage infiltration between control tissue and tumour tissue was then assessed using ImageJ software.

To test its oncolytic ability, HSV1716 was added to cultures of human myeloma cell lines at increasing multiplicity of infection (MOI). The virus was allowed to infect for 24 hours and then the media changed. Cell viability was then assessed at various times points ranging from 3 days post infection to 6 days post infection. 

Viability was determined cell counting and propidium iodide staining.

HSV1716 release from macrophages was tested by infecting macrophages with HSV1716 and then viral numbers in macrophage culture under hypoxic and normoxic was assessed for 72 hours post infection using standard viral titer assays.

To test the efficacy of macrophage-released HSV1716 to induce cell death, 72 hour conditioned medium from macrophages alone or following HSV1716 infection were added to two myeloma cell lines and viability assessed 4 days later. Following proof of principle in vitro studies a preliminary in vivo study was conducted. Human U226 cells were implanted via i.s into NSG mice as before. After 1 week of tumour growth mice were treated with the proteasome inhibitor bortezomib at 0.5 mg/kg every three days and a single dose of HSV1716 alone, HSV1716 with PMX5 and macrophages with HSV1716. After a further two weeks animals were sacrificed and bone density and bone lesion analyzed.

RESULTS

Macrophage and Hypoxia

Figure 2: Human xenografts induce robust macrophage recruitment. A) IHC for F4/80 shows a large number of macrophages infiltrating U266 and 1716 myeloma tumours. B) Image analysis shows a significant increase in macrophage numbers in U266 tumours when compared to control tissue. *p<0.05 vs act to control, bar = 25μm.

Figure 3: Human xenografts are positive for HIF-1α. IHC for HIF-1α showed the presence of large areas of hypoxia in myeloma tumours. Staining of control/healthy tissue showed no HIF-1α build up.

Figure 4: HSV1716 release from human macrophages 24 hours post infection. Increasing MOIs results in increased viral particle release and, under hypoxia, macrophages release significantly more virus than those in normoxia. ***p<0.01 (students t-test)

Conclusions

- Myeloma xenografts induce a significant increase in macrophage recruitment compared to normal tissue (Figure 2).
- Xenografts show a high degree of HIF-1α build up (Figure 3).
- HSV1716 is sequesereed and released by macrophages (Figure 4).
- HSV1716 infection results in a potent induction of myeloma cell death (Figure 5).
- Macrophages deliver functional HSV1716 to myeloma cells resulting in cell death (Figure 6).
- Preliminary analysis of murine models shows macrophage delivered virus improves myeloma outcomes significantly when combined with bortezomib.
- MM xenografts demonstrate a robust macrophage infiltration and are an appropriate model to study the development of therapeutic macrophages.
- HSV1716 induces cell death effectively in two myeloma cell lines and further investigation in other myeloma cell lines is under way.
- Preliminary data suggests that HSV1716 in combination therapies will improve therapeutic response over chemotherapy alone and further analysis is now warranted.