Why animal models of gene therapy have let glioma patients down: a systematic review and meta-analysis

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Malignant glioma

- Primary intrinsic brain tumour
  - Highly invasive
  - Difficult to excise
  - Chemo/radioresistant

- Poor prognosis
  - 14 months median survival in RCTs
    - Chemotherapy, radiotherapy, surgical debulking

- Few breakthroughs since radiotherapy in 1970s

http://radiopaedia.org/cases/glioblastoma-multiforme-6
Gene therapy for glioma

- Use of genes to treat disease, delivered to target cells by a vector
  - Typical approaches in glioma:
    - Kill cells (via prodrug)
    - Promote immune response

- Promising preclinical data

- Clinical trials discordant:
  - 2 unsuccessful phase-III RCTs
  - Variable results from smaller trials

Aims and hypothesis

- Systematic review and meta-analysis of preclinical literature testing gene therapies in experimental glioma

- Efficacy overstated in preclinical literature because of limitations in:
  1. External validity (experimental design)
  2. Internal validity (risk of bias)
Methods

**Systematic literature search:** Pubmed, EMBASE, Web of Knowledge

**Inclusion:** studies testing a gene therapy in an orthotopic rat/mouse model of glioma.
**Outcome:** median survival

**Data extraction:**
Features of gene therapy and glioma model
Study quality CAMARADES scale

**Analysis:** DerSimonian and Laird meta-analysis. Stratified meta-analysis, Egger regression, Trim and Fill analysis

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**Literature search**

- Pubmed, EMBASE, Web of Knowledge search: n=3860
- Duplicates excluded: n=578
- Inclusion criteria applied: n=3282
- Excluded: n=3074
- Data extracted for meta-analysis: n=208; 500 comparisons
- Excluded: 63 comparisons, 15 publications
- Publications included in meta-analysis: n=193; 427 comparisons, 6366 animals
Global efficacy estimate

Overall:

Gene therapy improved median survival by ratio 1.60 (95%CI 1.53-1.67)
Significant heterogeneity: $\chi^2=1522; I^2=72\%$

127 gene therapies
Most frequently reported:
• thymidine kinase
• IL-4
• IL-2
Gene therapy group did not account for heterogeneity (p>0.0019)
Risk of bias

- Median quality score 3/9 (IQR 3-4)
- 12.4% randomly allocated to groups
- 3.6% blinded assessment of outcome
- Neither quality score nor randomisation accounted for heterogeneity (p>0.0019)
Publication bias

- Funnel plot: asymmetry suggestive of publication bias

- Positive intercept on Egger regression \((p<0.001)\)

- Trim and fill – no studies added
Gene therapy factors

- Gene therapy vector:
  - Cells and virus-producing cells associated with greater efficacy than viruses (p<0.0019)

- Route of delivery:
  - No difference between local and systemic delivery (p>0.0019)
  - Heterogeneity between individual routes
Gene therapy factors

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  - Heterogeneity between individual routes

- Number of doses:
  - Greater efficacy with multiple doses (p<0.0019)
Disease model features

- **Species**
  - Greater efficacy in rats than mice ($p<0.0019$)

- **Immune status**
  - Immunocompromised animals common; associated with greater efficacy ($p<0.0019$)
Disease model features

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- **Tumour model**
  - 39 models reported, mostly from *in vitro* cultures
  - Only 1 patient-derived xenograft
  - Large differences in efficacy (p<0.0019)
Limitations

- Unable to directly stratify by individual gene therapies
  - Similar results seen in subset analysis of thymidine kinase

- Measures to reduce bias
  - Few high quality studies
  - Low prevalence of randomisation/blinding

- Limitations in construct validity
  - Immune compromised animals
  - Tumour models that behave differently to human disease
  - Efficacy of systemic therapy discordant with clinical experience
Conclusions

- Gene therapy remains a candidate therapy for glioma

- Observations that may contribute to translational failure:
  1. Internal validity (*low quality scores, low rate of randomisation/blinding, publication bias*)
  2. External validity (*route of delivery, vector and glioma model used*)
  3. Construct validity (*immunocompromised animals, tumour models*)

- Further studies based on these observations may help preclinical data better predict efficacy in humans
Thanks to…

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  - Aaron-Lawson McLean

- Western General Hospital, Edinburgh
  - Ian Whittle
  - Paul Brennan
  - Robin Grant

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Inclusion criteria

1. Single form of gene therapy
2. Rat or mouse model of glioma
3. Glioma cell line stated
4. Intracerebral tumour implantation
5. Median survival data presented
6. Number of animals stated in treatment and control groups
Quality scoring

1. Peer reviewed journal
2. Randomised group allocation
3. Blinded assessment of outcome
4. Sample size calculation
5. Compliance with animal welfare regulations
6. Statement of potential conflict of interests
7. Number of animals initially in each group
8. Explanation of animal exclusions
9. Statement of the number of tumour cells implanted
HSV-TK subset analysis

A

B

C

D

E

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Sample size calculations

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Number of animals needed per group

Statistical power (%) vs. Number of animals needed per group

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