Novel murine models of immune competent spontaneously-arising heterogeneous gliomas for evaluating the new classes of chemo-immunotherapeutics

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Limitations of current in vivo models for testing immune therapeutics

- Not immune competent.
- Cloned cell lines do not recapitulate the heterogeneous nature of intrinsic glioma.
- Cell lines perpetuated *in vitro* have significantly reduced immune suppression.

Therefore, models of spontaneously-arising heterogeneous tumors in immune competent animals are needed for the pre-clinical assessment of immune therapeutics.
The RCAS/\textit{tv-a} System

(Holland, et al, Genes Dev.15:1913, 2001)

Transgene \textit{Nestin} \textit{tv-a} Target Glioneuronal Progenitors

RCAS Vector LTR gag pol env \textbf{PDGF-B} LTR

TV-A (ALV-A Receptors)
Aberrant signals from RTKs, cytokines, NRTKS, PDGF-B

Dysregulated tyrosine kinase signalling

STAT3 activation

- Cyclins D1, D2, C-Myc
- p53

- Bcl-2, Bcl-xL, Mcl-1, Survivin

- MMP-3, MMP-9
- VEGF

- Immune surveillance

- Cell growth
- Survival
- Invasion/Metastasis
- Angiogenesis
- Immuno-suppression

Tumorigenesis/Tumor Progression

Adapted from Siddiquee et al, 2008
Hypothesis

(1) STAT3 and Bcl2 enhance tumor formation and grade in a PDGF-B dependent model of glioma.

(2) The gliomas formed in Ntv-a mice are immunosuppressive, and high-grade gliomas are more immunosuppressive relative to low-grade gliomas.

(3) Endogenously arising malignant gliomas can be used to test chemo-immunotherapeutics, such as the STAT3 inhibitor WP1066.
Experimental Protocol

*Ntv-a Mice

Inject RCAS vectors * into frontal lobe

90 day observation; experiment terminated

Histopathological & Immunological Analysis

* PDGF-B, Bcl2, STAT3, PDGF-B + Bcl2, or PDGF-B + STAT3
STAT3 and Bcl2 promote tumor formation, tumor grade, and increase mortality

![Graph showing tumor incidence and percent survival](image-url)

- **Total Tumor Incidence**:
  - Bcl2
  - STAT3
  - PDGF-B
  - PDGF-B+STAT3
  - PDGF-B+Bcl2

- **Tumor Grade**:
  - High Grade
  - Low Grade

- **Percent Survival**:
  - PDGF-B alone
  - PDGF-B+STAT3
  - PDGF-B+BCL2

Statistical significance:
- $P < 0.05$
- $P < 0.01$
Phenotypic features of \textit{de novo} malignant glioma in a PDGF-B + Bcl2 mouse

- **Low-grade**
  - GFAP
  - PDGF-B
  - Bcl-2
  - Magnification: 50 X

- **High-grade**
  - Magnification: 50 X

- **Infiltration**
  - Magnification: 100 X

- **Vascular proliferation**
  - Magnification: 400 X
Intratumoral pSTAT3 Expression

High-grade

Low-grade

**Intratumoral pSTAT3 Expression**

**(P < 0.01)**

**Died**

N=8

**Survivor**

N=4

(P < 0.01)
**Intratumoral Macrophage Infiltration**

High-grade

Low-grade

![Images of histological sections showing macrophage infiltration in high-grade and low-grade tumors.](100 X 400 X)

![Histogram showing the percentage of macrophages in high-grade and low-grade tumors.](P < 0.05)

![Bar chart comparing the survival rates of died and survivor groups.](P < 0.01)

- **PDGF-B+Bcl2 High Grade**
- **PDGF-B+Bcl2 Low Grade**

Survival (days)

% Macrophages

N=8

N=4
Intratumoral FoxP3+ Treg Infiltration

High-grade

Low-grade

% FoxP3+ Tregs

Survival (days)

PDGF-B+Bcl2 High Grade
PDGF-B+Bcl2 Low Grade

Died
Survivor

% FoxP3+ Tregs

0.0 2.0 4.0 6.0 8.0

0 20 40 60 80 100

Survival (days)
The utility of testing immunotherapeutics in heterogeneous immunocompetent model

![Graph showing percent survival over days post the construct injection, with lines for Control and WP1066 treatments.](image1)

![Bar graph showing percentage of pSTAT3 positive cells and macrophages, with significance indicated by * (P < 0.05).](image2)
Summary

• STAT3 and Bcl2 significantly enhanced tumor formation and grade in a PDGF-B dependent murine model of glioma.

• Intratumoral STAT3 expression and macrophage infiltration correlated with tumor grade and have prognostic significance, similar to studies of human gliomas.

• The STAT3 inhibitor WP1066 decreased both intratumoral STAT3 expression and macrophage infiltration, which correlated with increased survival.
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