



Combining *Listeria monocytogenes* Injection with TGF- β 1 Knockdown Provides a Synergistic Effect on Treatment of Subcutaneous Liver Tumors



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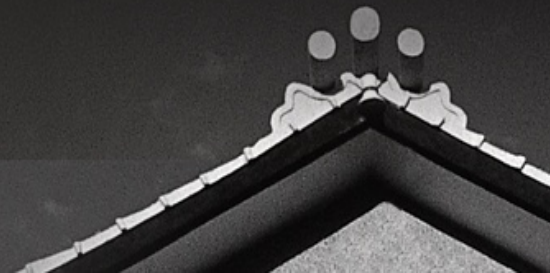
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We have no relevant financial relationship to disclose.

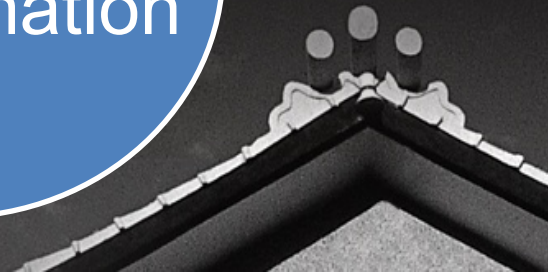
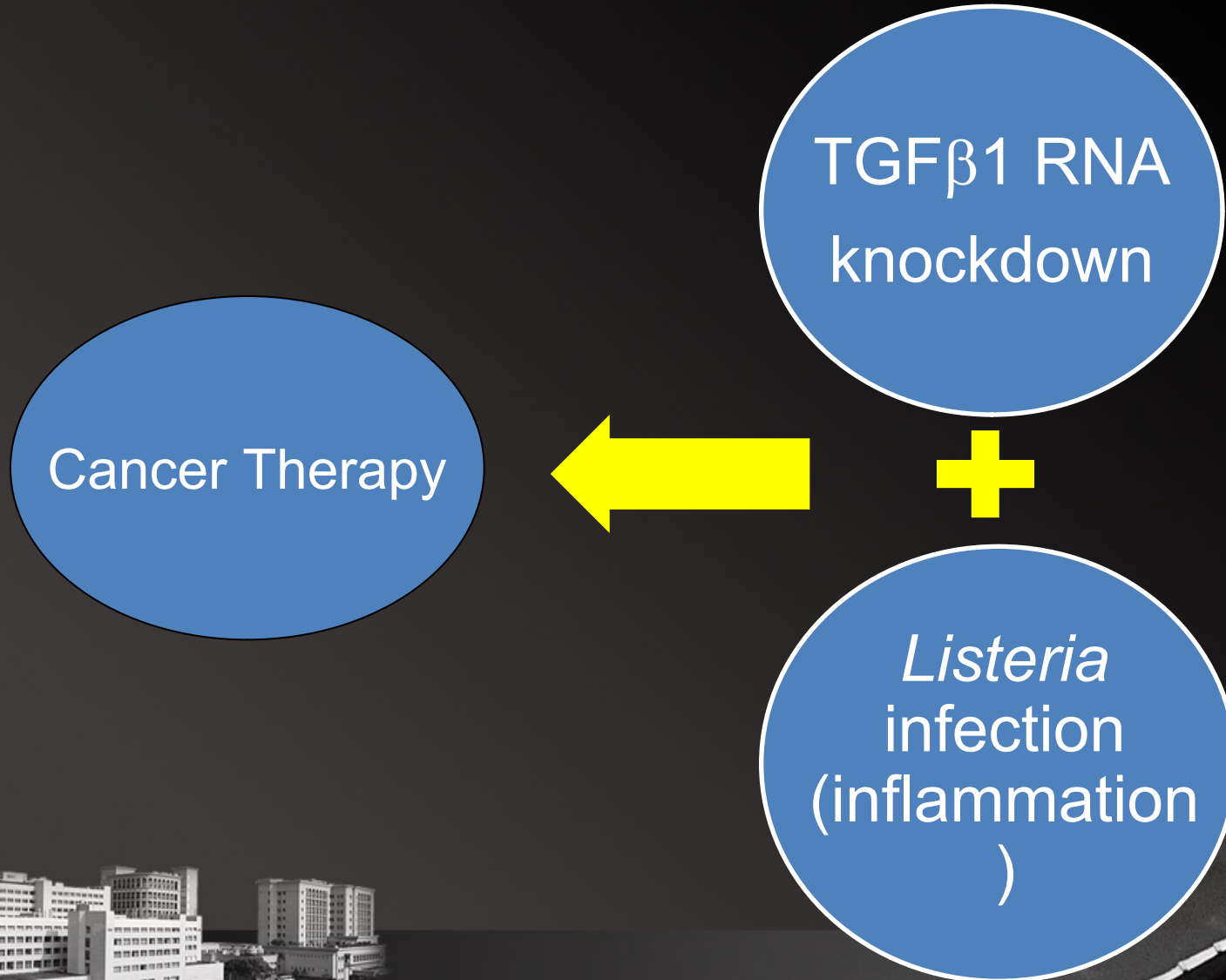


Introduction

- Cancer therapy: reduce immunosuppression (Transforming Growth Factor-Beta1, **TGF- β 1**) and activate immunity (***Listeria monocytogenes*** inducing inflammation)
- Material:
 - Animal: syngeneic mice (Balb/c mice)
 - Cancer cell: hepatocellular carcinoma (**BNL 1ME A.7R.1**) of Balb/c mice
- Methods: TGF- β 1 and *Listeria monocytogenes* for HCC treatment in mice



Knockdown and *Listeria* Infection



Listeria monocytogenes Infection

Day 1

BNL tumor cells

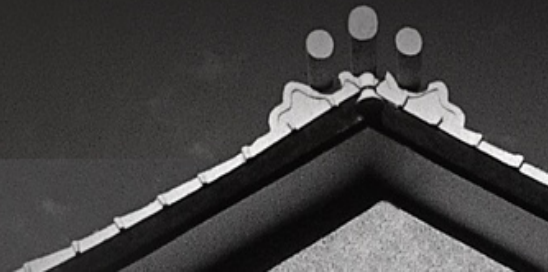
BNL tumor cells + *L. monocytogenes*

subcutaneous Injection into
Balb/c mice

Day 70 Tumor free mice

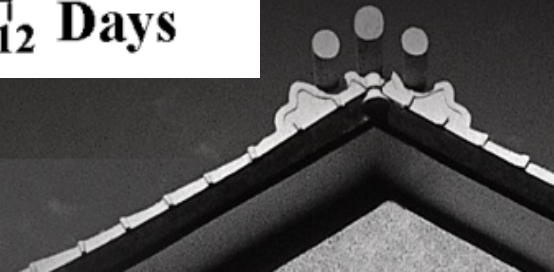
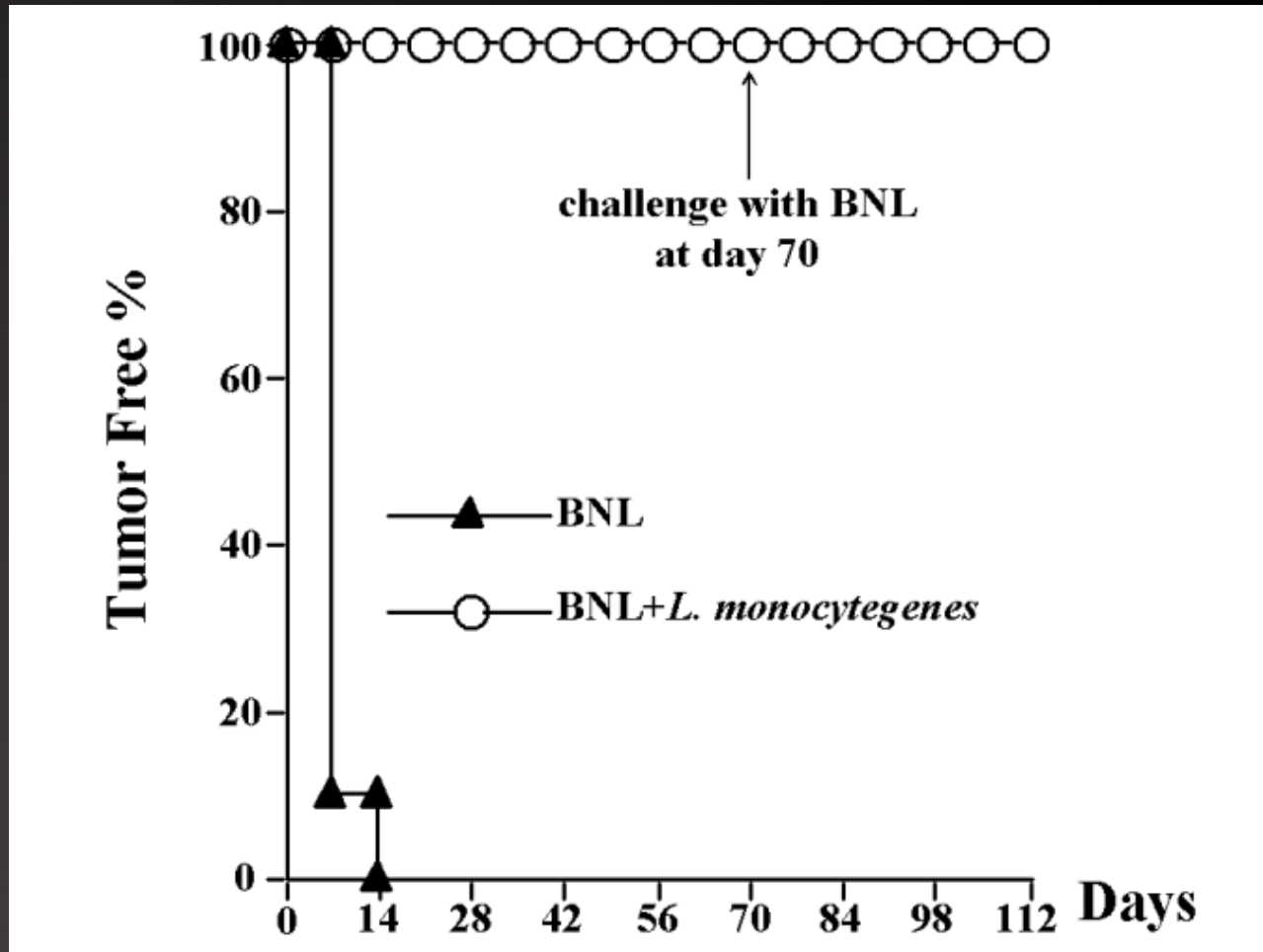
Re-inject BNL tumor cells

Tumor Free (%)



Anti-Tumor Immunity by

Listeria monocytogenes Infection



Inhibit Tumor Growth by



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Listeria monocytogenes Infection

Day 1

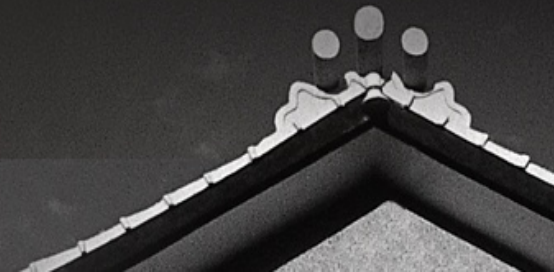
Subcutaneous injection with
BNL tumor cells

Day 7

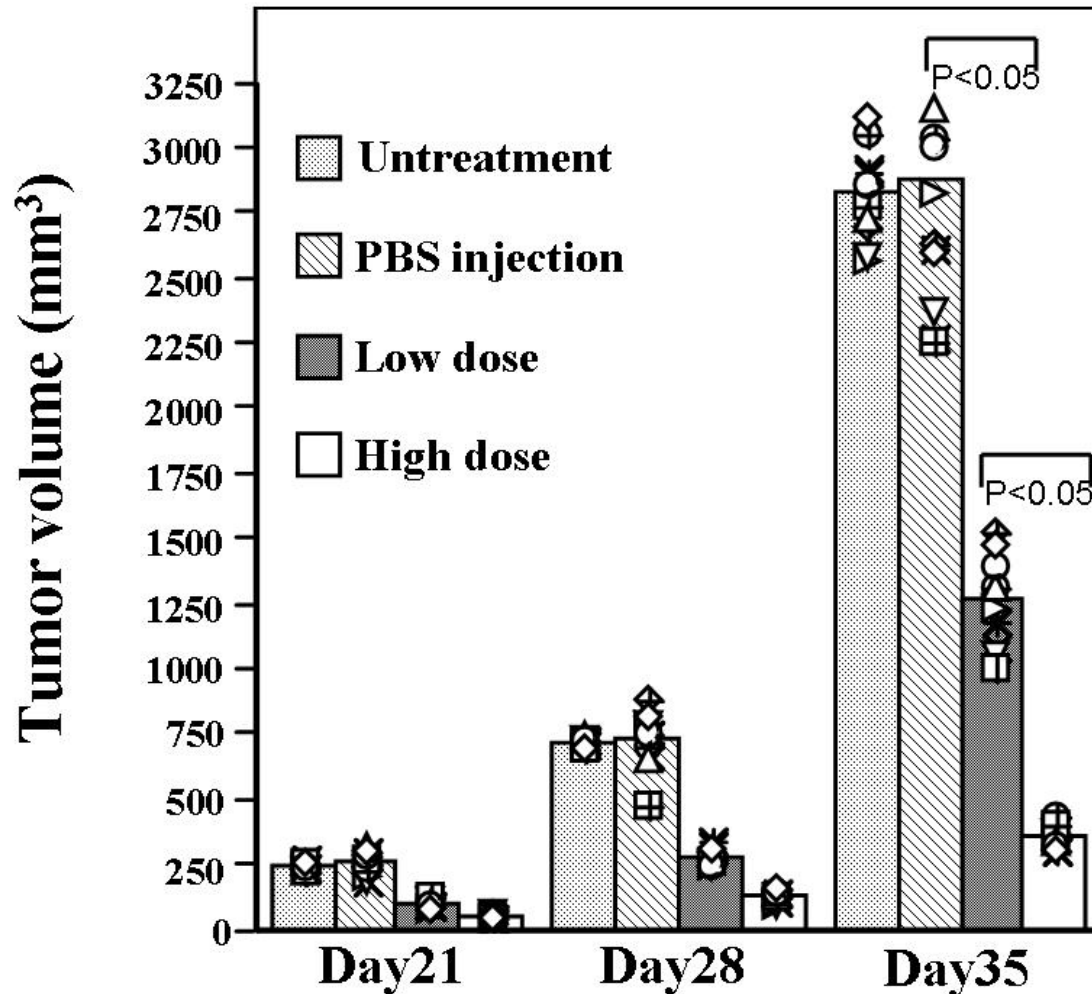
Tumor size
3 – 5 mm

1. PBS injection
2. High dose *L. Monocytogenes*
(5×10^4 pfu)
3. Low dose *L. monocytogenes*
(2.5×10^4 pfu)

Measure tumor size



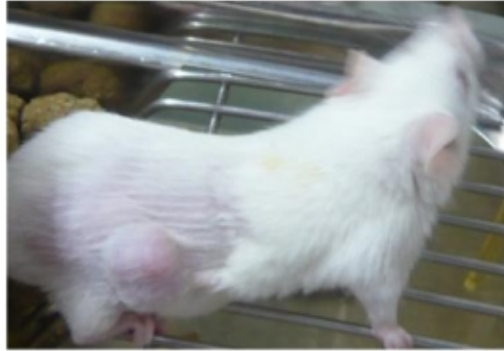
Listeria monocytogenes Infection



Inhibit Tumor Growth by

Listeria monocytogenes Infection

Untreatment



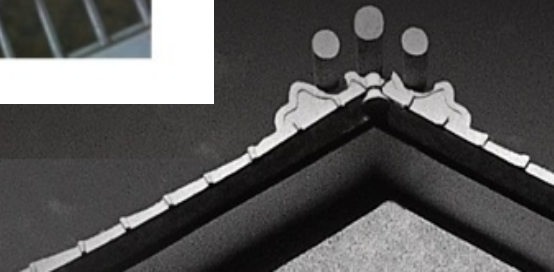
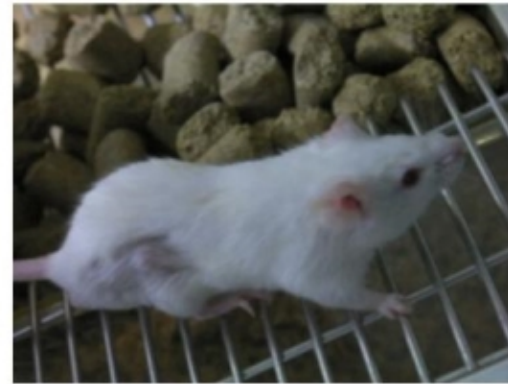
PBS injection



Low dose

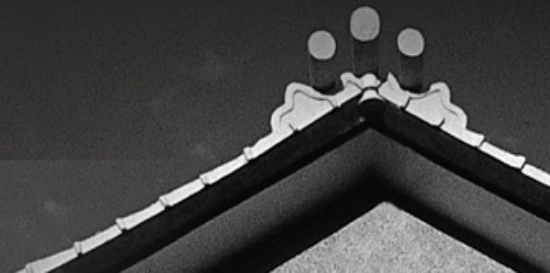


High dose



TGF β 1 Knockdown Cell line

- RNA interference (RNAi) can specifically and effectively direct homologous-dependent post-transcriptional gene silencing.
- In order to transfer RNA interference to tumor cell, we chose an recombinant retroviral vector as a delivery vehicle.



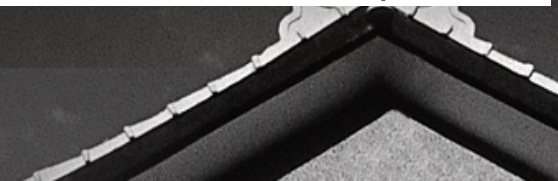
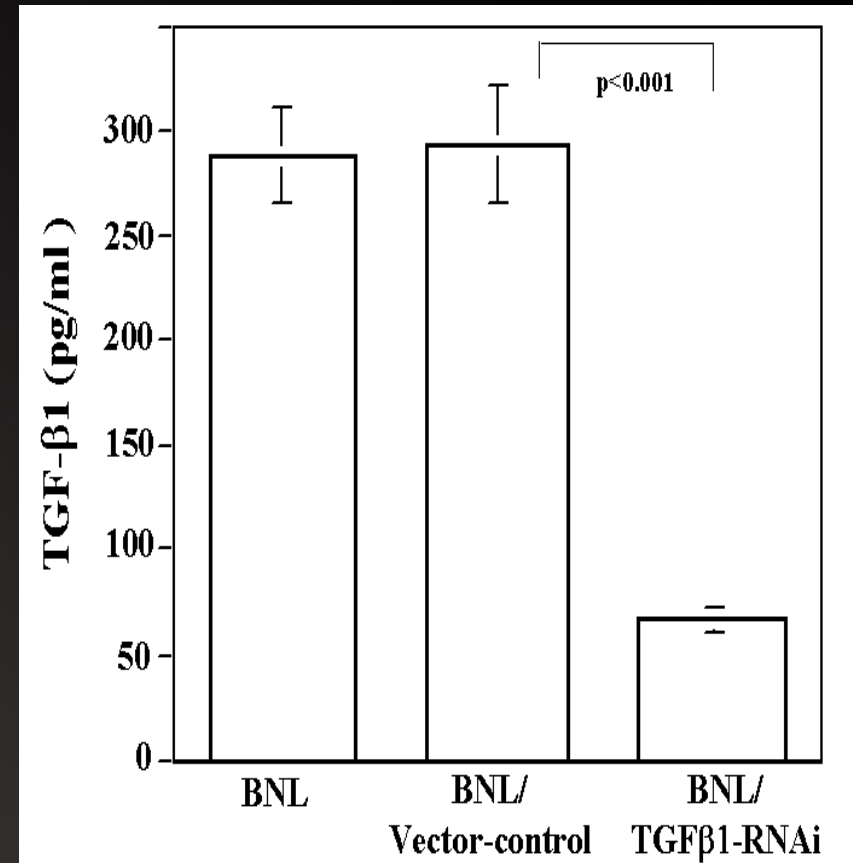
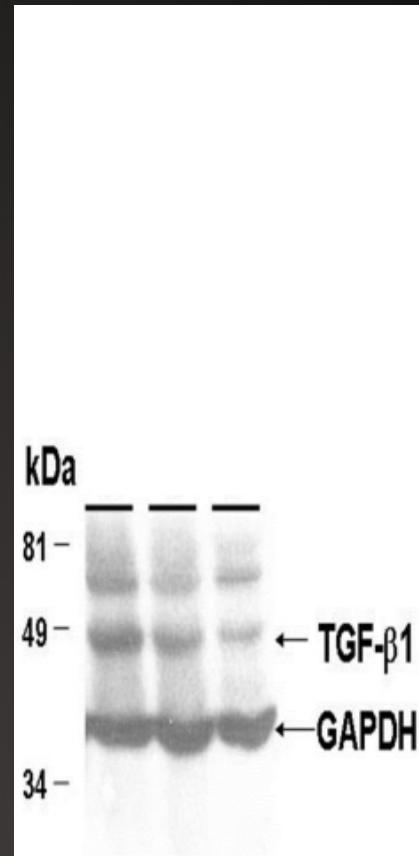
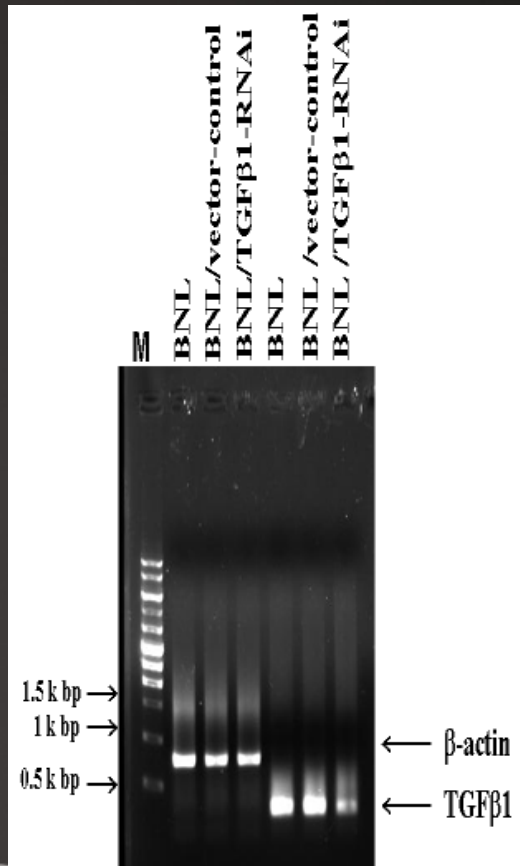
Knockdown TGF- β 1 by

RNA Interference

RT-PCR

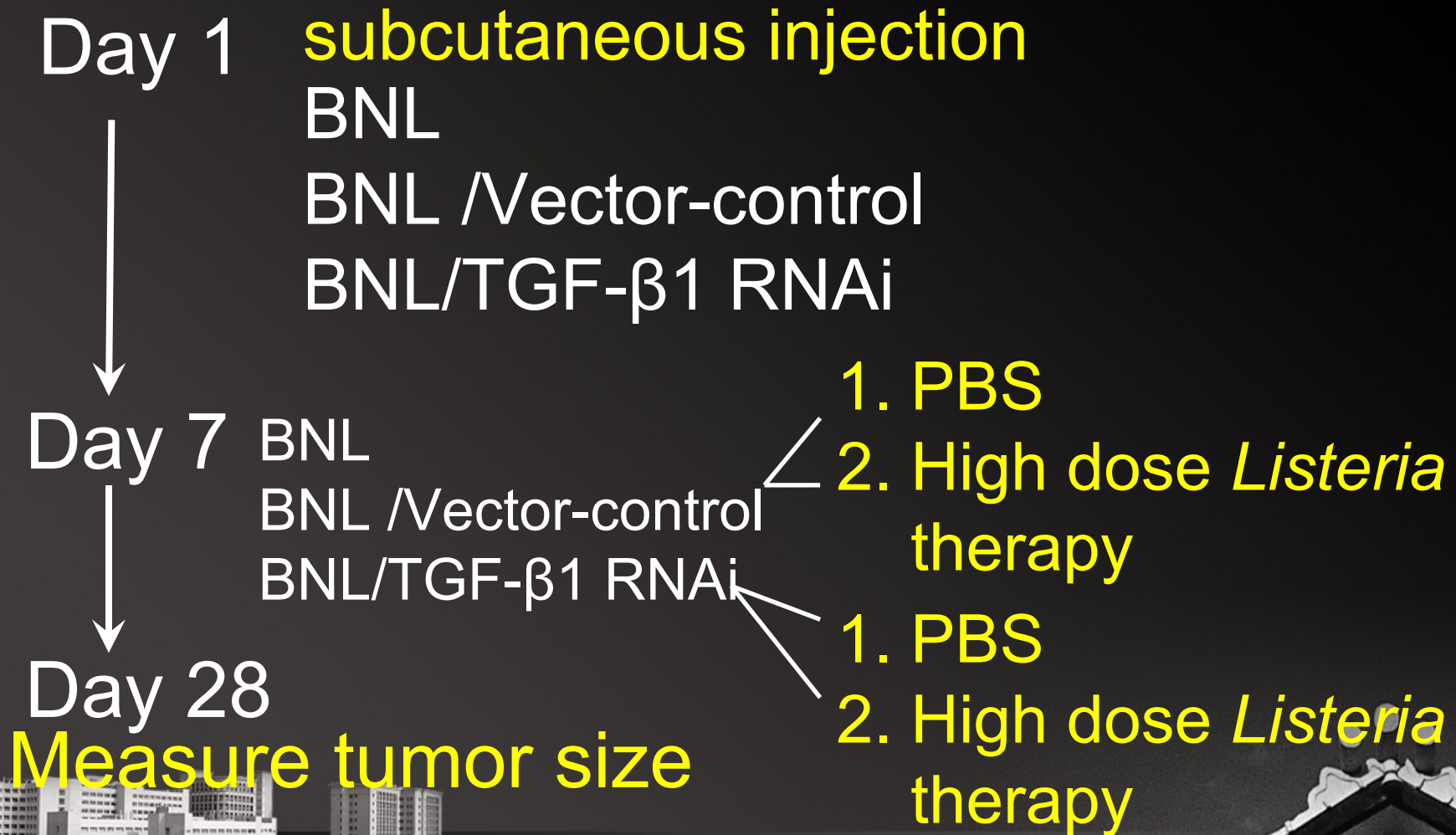
Western blotting

ELISA



Synergic Anti-tumor Effects of

TGF- β 1 Knockdown and *Listeria* Infection



Synergic Anti-tumor Effects of

TGF- β 1 Knockdown and *Listeria* Infection

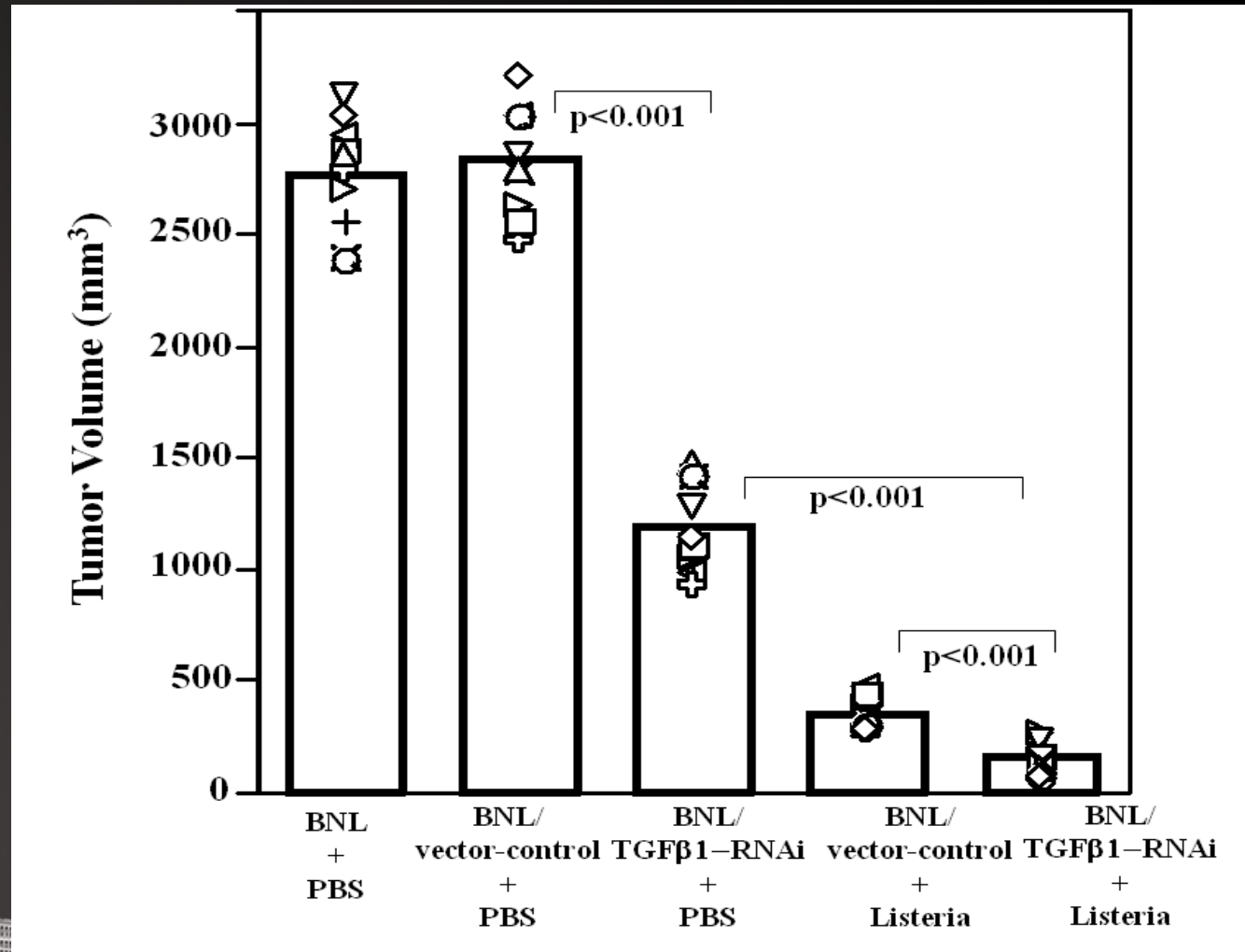


Table 1. Cellular infiltrates at subcutaneous tumor sites

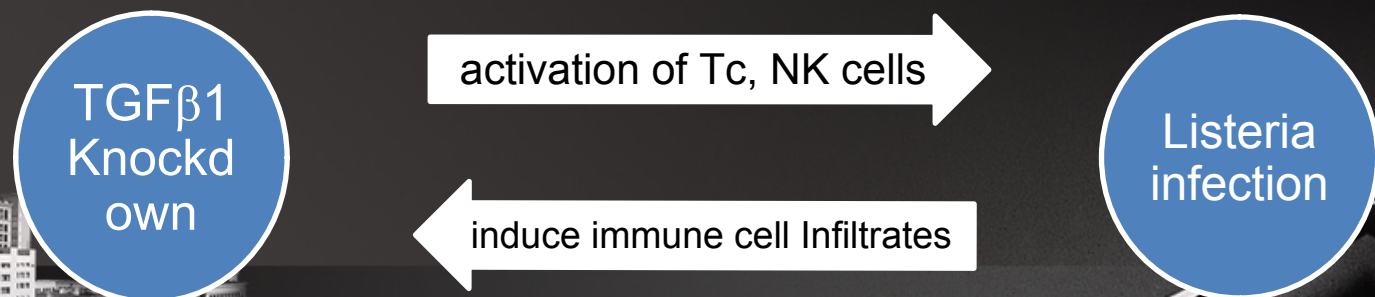
Treatments	Effectors present on day 7 after Listeria injection			Effectors present on day 14 after Listeria injection		
	Gr	CD4	CD8	Gr	CD4	CD8
BNL+ PBS	^a + / -	+ / -	+ / -	+ / -	+ / -	+ / -
BNL/vector- control + PBS	+ / -	+ / -	+ / -	+ / -	+ / -	+ / -
BNL/TGFβ1 RNAi + PBS	+	+	+	++	+	++
BNL/vector- control + LM treatment	+++	+	+	++	++	+
BNL/TGFβ1 RNAi + LM treatment	+++	+	+	+++	+++	++

Abbreviations: Gr, granulocytes; CD4, CD4⁺ T-cells; CD8, CD8⁺ T-cells

^aCellular infiltrates were examined by immunohistochemical staining (100×). The levels of cells were determined by averaging the cell numbers from five independent fields. (+ / -), Cell numbers were smaller than 5 cells/mm²; (+) 5-20 cells/mm²; (++) >20 cells/mm².

CONCLUSION

- Reduce TGF- β 1 protein by **TGF- β 1 RNAi** significantly
- *L. monocytogenes* makes inflammation and reduce tumor volume
- Combination of **TGF- β 1 RNAi** and *L. monocytogenes* achieve **very significant** tumor volume reduction
- Include two phases of immune response:
 - early T cell-independent phase (**Granulocyte**)
 - late T cell-dependent phase (**CD4+ and CD8+**)



Thanks for Your Reading



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and

Welcome to Taiwan

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We are here.

