

**Radiofrequency Ablation Combined
with huKS-IL2 Immunocytokine (EMD
273066) Results in an Enhanced
Antitumor Effect Against Murine Colon
Adenocarcinoma**

**Erik Johnson, MD
University of WI- Madison
Paul Sondel Lab
Depts. of General Surgery, Pediatrics, and Human Oncology**

Epidemiology: Colon Cancer

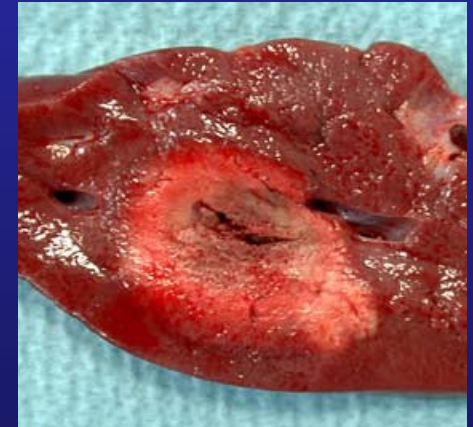
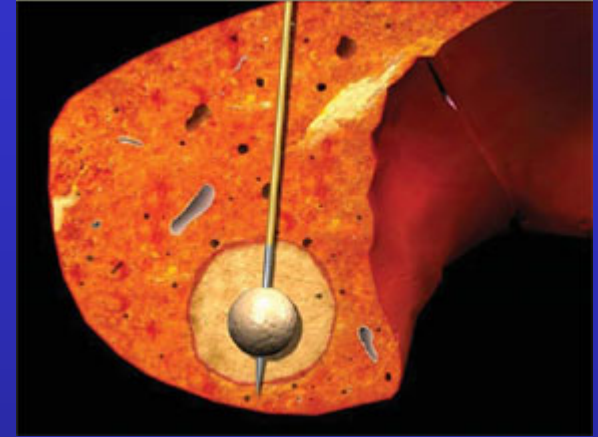
- In 2008: 148,000 new diagnoses of colorectal adenocarcinoma ¹
 - 20% (~30,000 patients) present with metastatic disease
 - Systemic recurrence after curative resection: 17-30% ²
 - Liver is most common site of metastasis and recurrence
- Treatment of liver metastases:
 - Surgery is most effective
 - 80-90% of eligible patients are unresectable, due to:
 - Tumor location near key vascular or biliary structures
 - Multifocal disease without enough residual liver for survival
 - Patient comorbidities
 - Treatment options: chemotherapy, ablation (microwave, cryoablation, RFA)

1. NCI Seer database (seer.cancer.gov)

2. Platell, C. Int J Colorectal Dis, 2007; 22: 1223-1231

Radio-frequency Ablation (RFA)

- Treatment modality for unresectable primary or metastatic tumors
 - Most commonly used for liver tumors
 - Increasing applications in primary tumors of breast, thyroid, lung
- Mechanism:
 1. Probe inserted into tumor and attached to RF generator
 2. Energy transmitted to non-insulated probe tip
 3. Results in thermal coagulative necrosis of tumor cells



RFA: Limitations

Efficacy is limited by size:

Contraindicated in:

- **Colorectal cancer liver metastases >4cm diameter**
- **HCC lesions >5cm**

Benefit: modest survival advantage

- **Survival 36 months with lesions 3-5 cm, vs. 11-14 months with chemotherapy using historic controls¹**

The problem with RFA: long term outcome

- **HCC and colorectal adenocarcinoma in the liver: up to 50% local and systemic recurrence ^{2, 3}**

Overall, RFA is good but it could be much better

1. Berber, et al. Predictors of Survival after Radiofrequency Thermal Ablation of Colorectal Cancer Metastases to the Liver: A Prospective Study. JCO; 2005, 23(7): 1358-64.
2. Sipperstein, et al. Local recurrence after laparoscopic radiofrequency thermal ablation of hepatic tumors. Annals of Surgical Oncology, 1999; 7(2): 106-113.
3. Curley, et al. Radiofrequency ablation of malignant liver tumors. Annals of Surgical Oncology, 2003; 10(4): 338-347.

RFA and the Immune System

- Prior studies indicate that RFA may have an additional effect: immune stimulation

[CANCER RESEARCH 63, 6496–6500, October 1, 2003]

Activation of Tumor-specific T Lymphocytes by Radio-Frequency Ablation of the VX2 Hepatoma in Rabbits¹

Thädaus Till Wissniowski,² Johannes Hünsler,² Daniel Neureiter, Markus Frieser, Stefan Schaber, Birgit Esslinger, Reinhard Voll, Deike Strobel, Eckhart G. Hahn, and Detlef Schuppan³

Radiofrequency Thermal Ablation of Hepatocellular Carcinoma Liver Nodules Can Activate and Enhance Tumor-Specific T-Cell Responses

Alessandro Zerbini,¹ Massimo Pilli,¹ Amalia Penna,¹ Guido Pelosi,¹ Claudia Schianchi,¹ Atim Molinari,¹ Simona Schivazappa,¹ Carlo Zibera,² Francesco F. Fagnoni,² Carlo Ferrari,¹ and Gabriele Missale¹

- Healing ablated tissue results in an intense inflammatory response
- Formerly hidden tumor antigens may be exposed

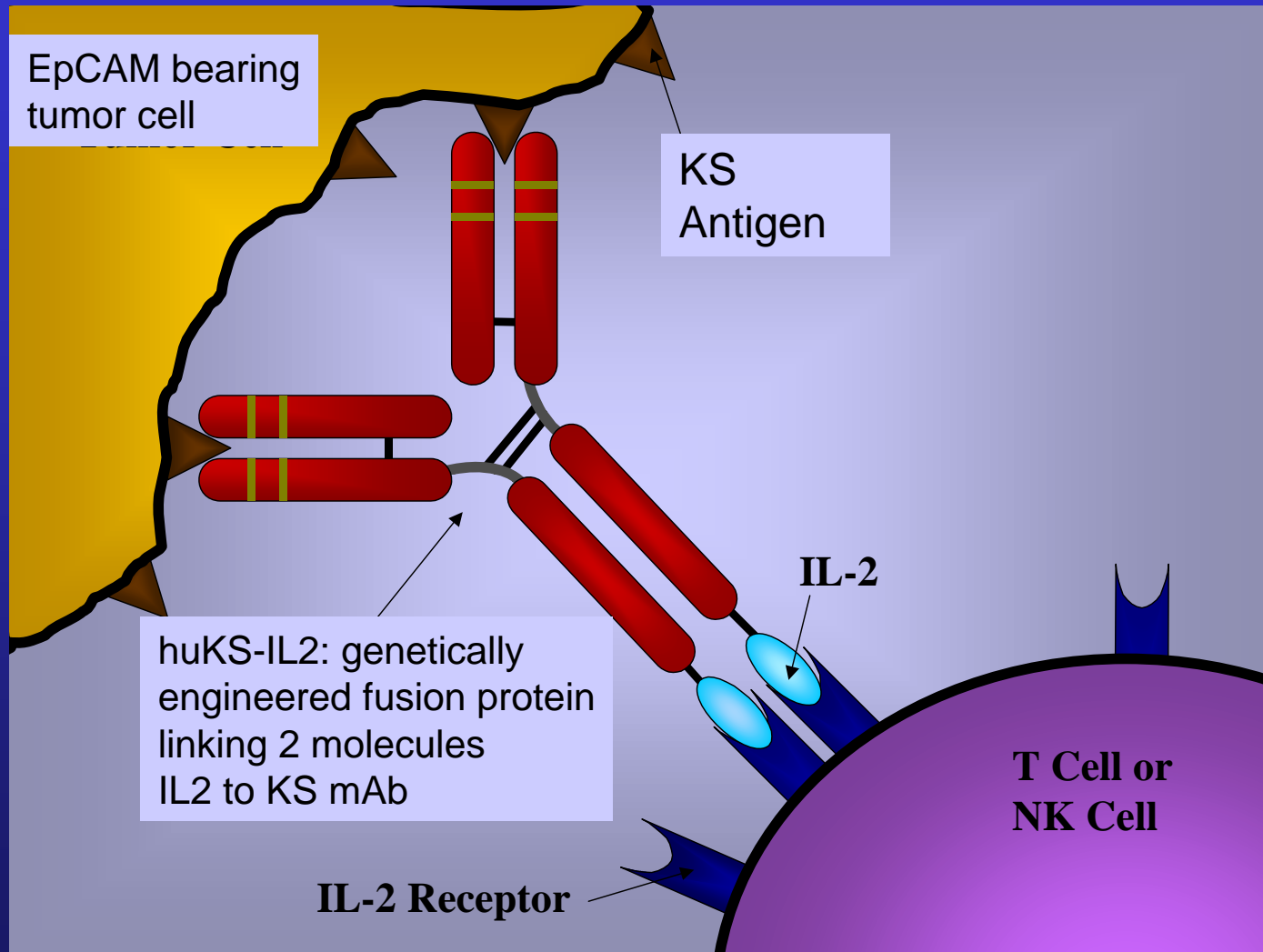
Experimental Purpose: combine RFA with a potent immune therapy that is effective in IT injections ¹

Can combination therapy produce augmented anti-tumor benefit in vivo?

- 1. Tumor growth and animal survival**
- 2. Complete tumor resolution**
- 3. Effect on distant, untreated tumors**
- 4. Induction of anti-tumor memory response**

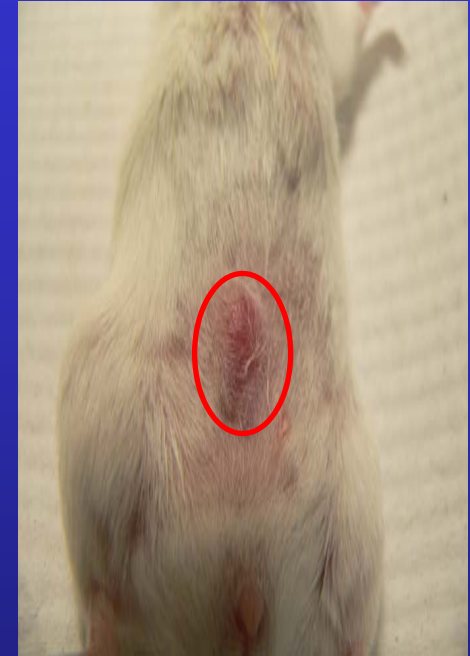
1. Johnson EE, Lum HD, Sondel PM. *Can Immunol Immunother*, 2008; 57: 1891-902

huKS-IL2: Mechanism of Action

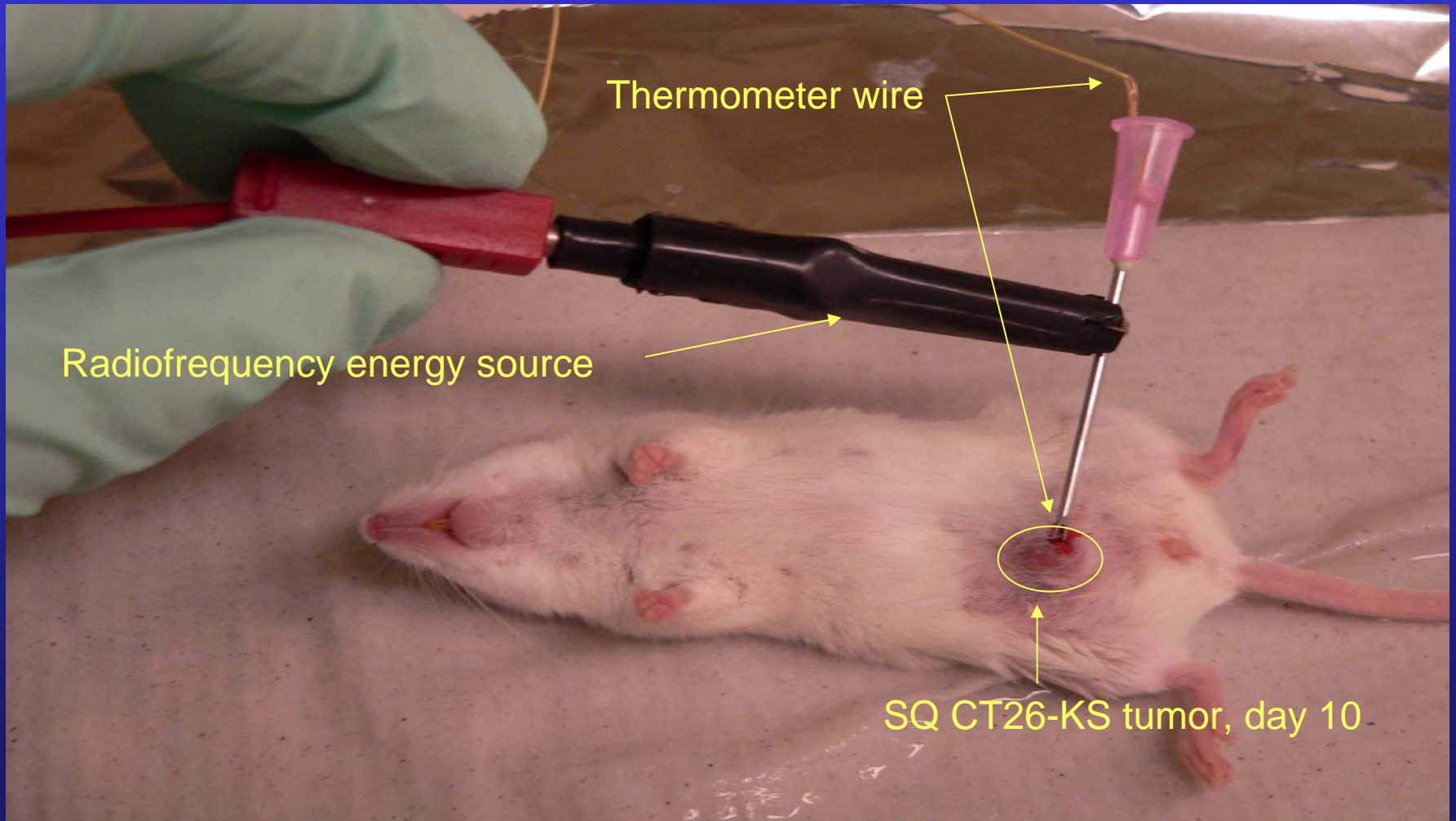


Methods: Tumor and Mouse System

- Balb/c mice
- CT26-KS colon adenocarcinoma, subcutaneous placement on the abdomen
 - Advantage of subcutaneous tumor:
 - RFA without surgery
 - Easily follow tumor growth
 - Treat via intra-tumoral injection
- CT26: Balb/c derived colon cancer
 - KS: human EpCAM antigen (epithelial derived)
- Treated with RFA at 90°C for 25-30 seconds
 - To simulate clinical tumor recurrence, the tumor was partially ablated
- Immune therapy via IT injection

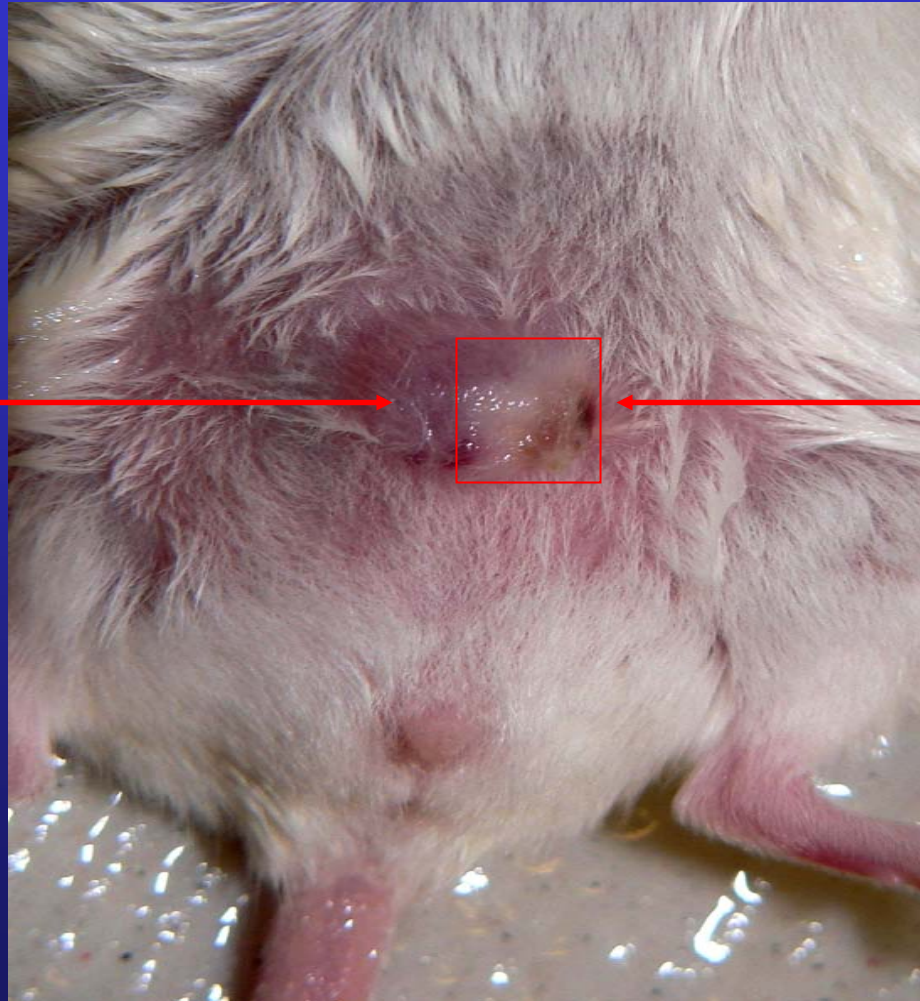


Methods: Tumor Ablation



Methods: Partial Tumor Ablation

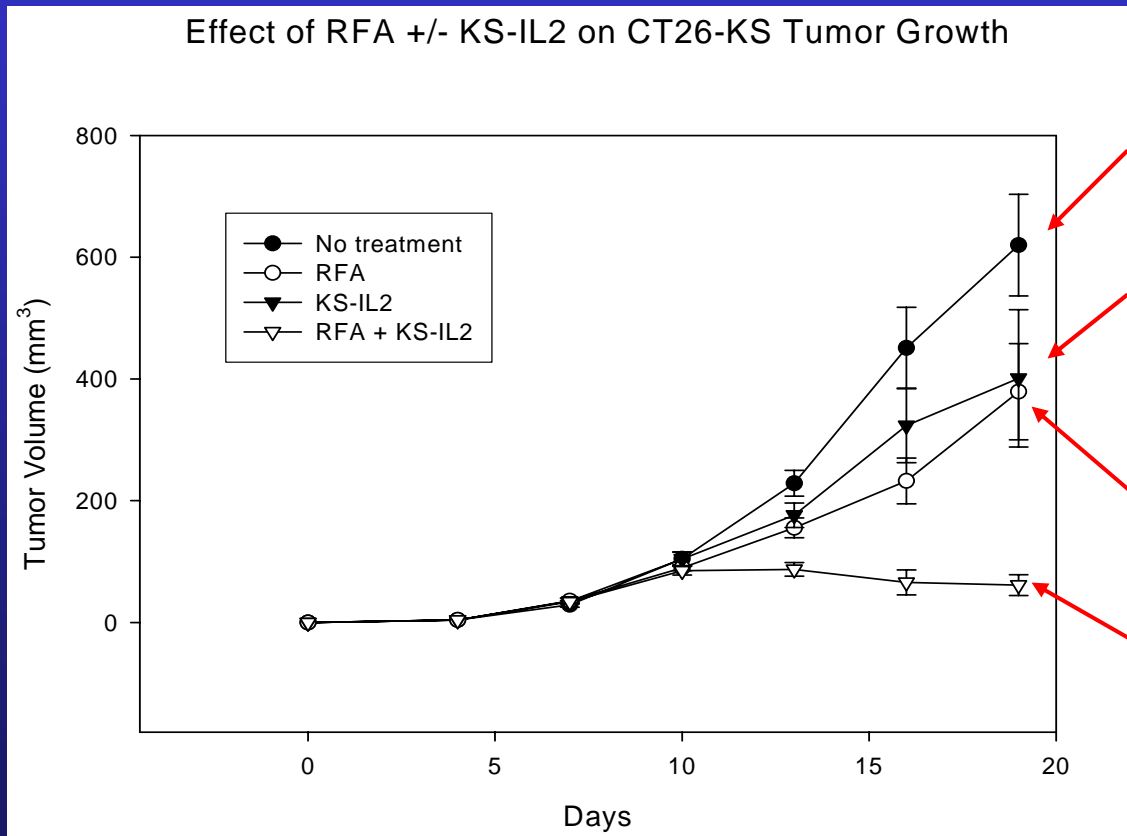
Remaining viable tumor for recurrence



Zone of direct thermal injury

Results: Effect of RFA +/- huKS-IL2 on Tumor Growth

4 treatment groups, 8-10 mice per group



Untreated

huKS-IL2 IT, 15 ug per dose,
5 doses on days 11 -15

RFA alone:
25 sec on day 11

RFA + huKS-IL2 ($p < 0.001$)
Same dose of RFA with the
addition of huKS-IL2

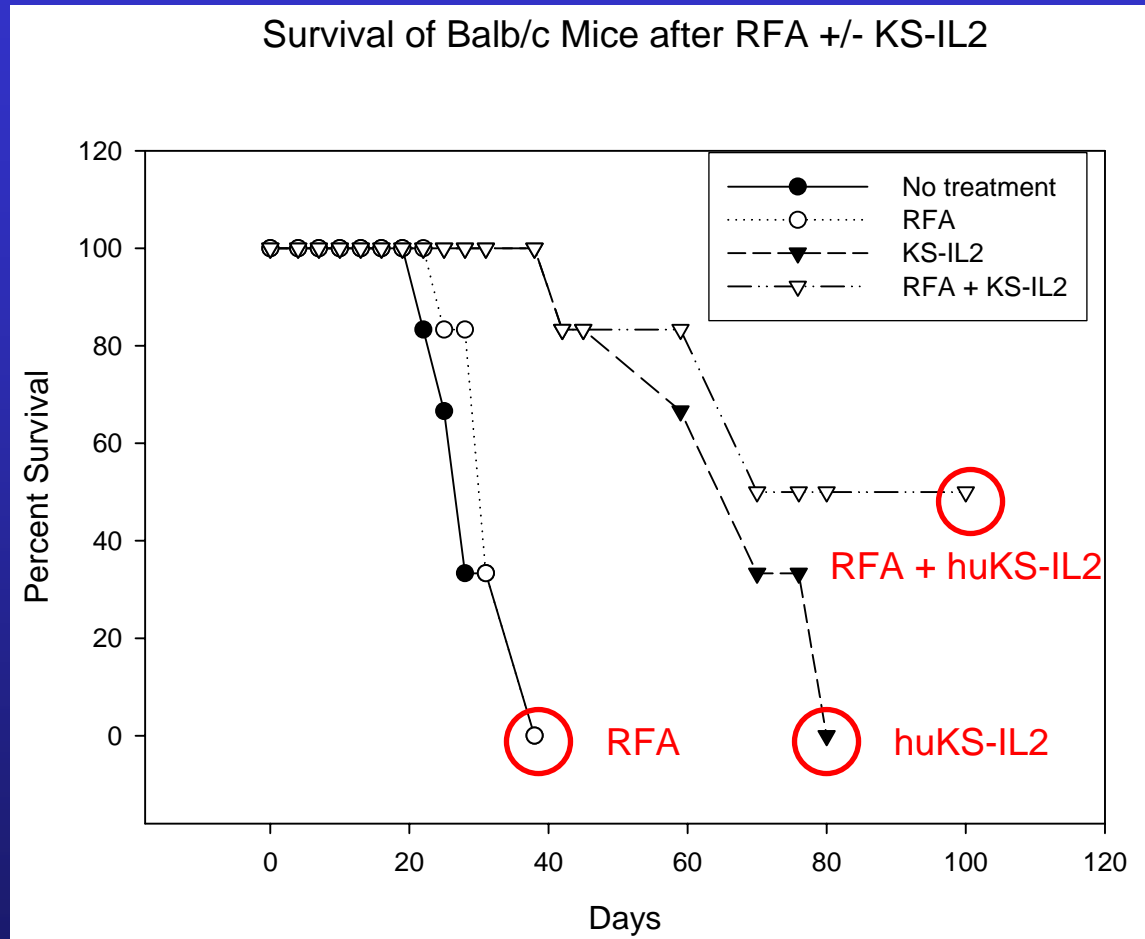
Conclusion: huKS-IL2 alone provides some benefit in slowing tumor growth. When combined with RFA, results in greatest effect (5/10 complete resolution, 0 in other groups)

Tumor Resolution

- RFA + huKS-IL2 not only slows tumor growth, but also may result in complete tumor resolution of the CT26-KS tumor

Treatment	Number tumor-free: synergistic conditions	All Experiments (3)
No treatment	0 / 14	0 / 26
RFA alone	0 / 14	5 / 26 p = 0.051 vs. no treatment p = NS vs. KS-IL2 alone
huKS-IL2 alone	0 / 14	3 / 26 p = NS vs. no treatment p = NS vs. RFA alone
RFA + huKS-IL2	8 / 16 p = 0.002 vs. all groups	14 / 28 p < 0.001 vs. no treatment p = 0.024 vs. RFA alone p = 0.003 vs. huKS-IL2 alone

Survival after RFA +/- huKS-IL2



Conclusion: RFA + huKS-IL2 results in significantly enhanced survival compared to untreated animals ($p < 0.001$), RFA alone ($p < 0.001$), and huKS-IL2 alone ($p = 0.002$)

Does complete tumor resolution of the initial tumor have anything to do with the immune system?

– To answer this we also tested:

- The effect of RFA with huKS-IL2 on distant tumors**
- Tumor rechallenge experiments**

Effect of RFA and huKS-IL2 on Treated and Distant CT26-KS Tumors

Method: CT26-KS tumors were implanted on both the abdomen and the flank; only the abdominal tumor was treated with RFA, huKS-IL2, or RFA and huKS-IL2

Treatment Group	Resolved abdominal tumor	Resolved untreated flank tumor	Proportion completely tumor-free
No treatment	1 / 15	0 / 15	0 / 15
RFA	5 / 15	0 / 15	0 / 15
huKS-IL2	7 / 15 p = 0.035 vs. NT	8 / 15 p = 0.002 vs. NT p = 0.002 vs. RFA	7 / 15 p = 0.006 vs. NT p = 0.006 vs. RFA
RFA + huKS-IL2	14 / 16 p < 0.001 vs. NT p = 0.003 vs. RFA p = 0.023 vs. huKS-IL2	13 / 16 p < 0.001 vs. NT p < 0.001 vs. RFA p = 0.14 vs. huKS-IL2	13 / 16 p < 0.001 vs. NT p < 0.001 vs. RFA p = 0.044 vs. huKS-IL2

Immune Memory: Tumor Rechallenge

Mice which resolved their CT26-KS tumor were challenged simultaneously with CT26 (1st exposure) and CT26-KS (2nd exposure) on opposite flanks

Shown Below: naïve animal (control)



Results: Tumor Rechallenge

Group	CT26-KS tumor-free (2 nd exposure)	CT26 tumor-free (1 st exposure)
Naïve control	0 / 4	0 / 4
RFA alone	5 / 5	1 / 5 p = NS vs. control
KS-IL2 alone	3 / 3	3 / 3 p = 0.004 vs. control p = NS vs. RFA alone
RFA + KS-IL2	9 / 9	7 / 9 p = 0.004 vs. control p = 0.018 vs. RFA alone p = NS vs. huKS-IL2 alone

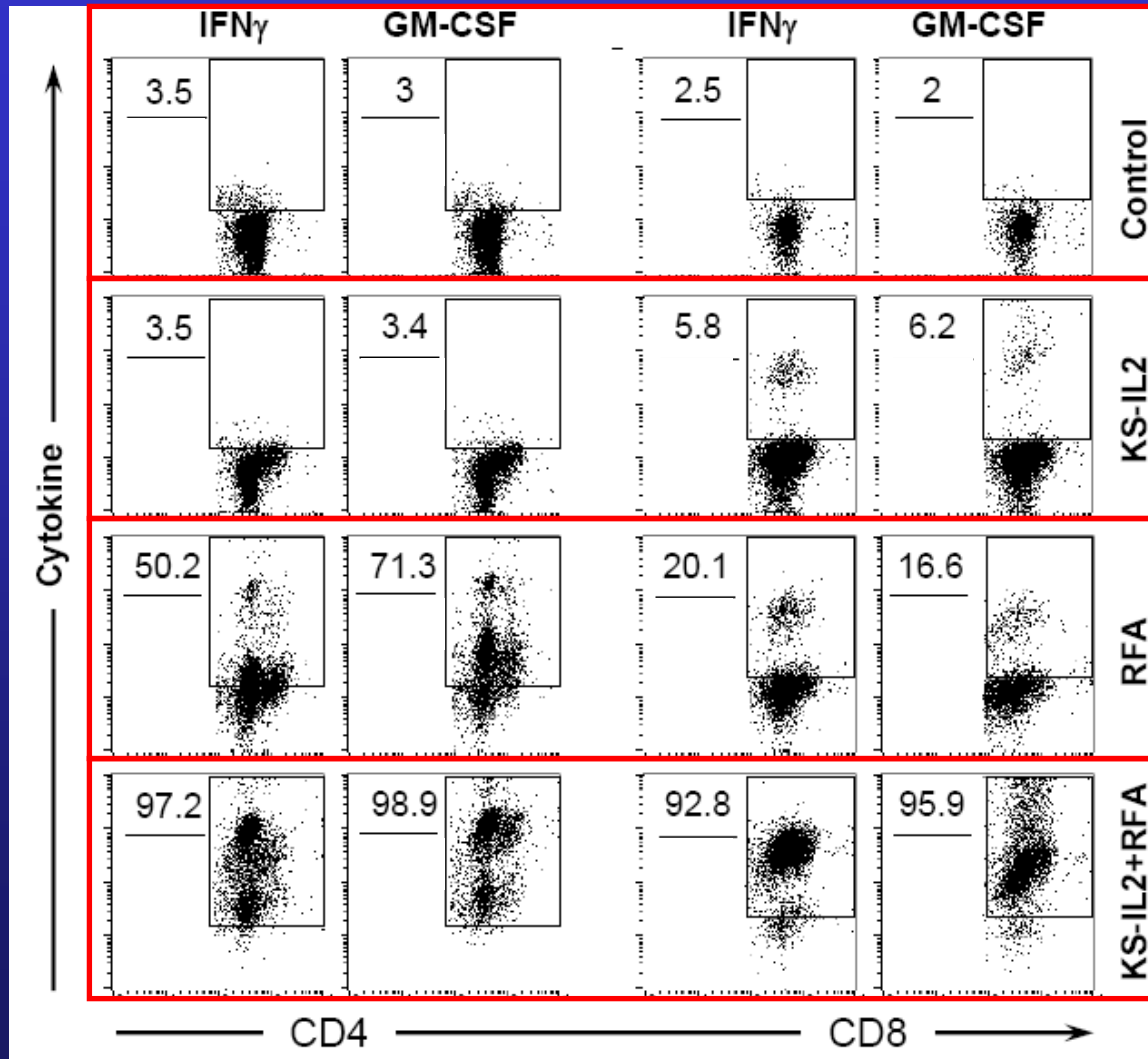
Conclusion:

1. All animals which initially resolved the CT 26-KS tumor had memory, from either RFA, KS-IL2 or combined therapy
2. The addition of immune therapy (KS-IL2) enhanced the immune response to shared tumor antigens between the two cell lines, separate from the potent KS antigen

The Immune Response to RFA

- Does ablation serve only to debulk the tumor, which allows huKS-IL2 to demonstrate efficacy?
- T cell cytokine expression assay:
 - CT26-KS tumor implantation
 - Mice tumor-free with: complete ablation, tumor excision, or partial ablation combined with huKS-IL2
 - Monitored for 30 days for recurrence
 - Sacrificed, spleens harvested
 - Cultured with irradiated CT26-KS or Meth A sarcoma cells for 5 days

Proportion of CD4 or CD8 Cells with Cytokine Expression



Summary of Flow Data: 3-5 experiments

Average percentage of CD4 or CD8 cells with cytokine expression

Treatment Group	CD4 IFN- γ	CD4 GM-CSF	CD8 IFN- γ	CD8 GM-CSF
Naïve	2.5 +/- 0.5	2.5 +/- 0.5	4.0 +/- 0.6	3.9 +/- 0.6
Surgery	9.3 +/- 4.1	9.9 +/- 5.3	7.7 +/- 1.7	17.8 +/- 8.2
huKS-IL2	19.0 +/- 7.7	44.0 +/- 20.9	30.0 +/- 12.3	54.6 +/- 25.3
RFA	40.0 +/- 14.0	46.5 +/- 16.8	29.9 +/- 12.5	31.2 +/- 10.5
RFA + huKS-IL2	69.0 +/- 9.3	84.0 +/- 6.2	80.8 +/- 4.7	91.0 +/- 2.5

Conclusions:

1. Although both surgery and RFA serve to debulk the tumor, there are differences in the immune response to tumor antigens on a cellular level
2. Although huKS-IL2 is effective as a single agent, the response to combination therapy with RFA + huKS-IL2 is more robust
3. Overall, treatment with RFA and huKS-IL2 results in the most antitumor response

Conclusions:

- **Although somewhat effective, RFA can be improved**
- **The addition of huKS-IL2 therapy to RFA:**
 - **Results in a synergistic antitumor response**
 - **Primary tumor growth**
 - **Overall survival**
 - **Complete tumor resolution**
 - **Induces a systemic antitumor immune response**
 - **Effective against distant tumors (potential benefit in the setting of micrometastatic disease)**
 - **Creates an immune memory response to subsequent tumor challenges, including CT26 tumors**
 - **These two therapies appear to work synergistically**
 - **Overall may be a promising therapeutic modality for the treatment of stage 4 disease**

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