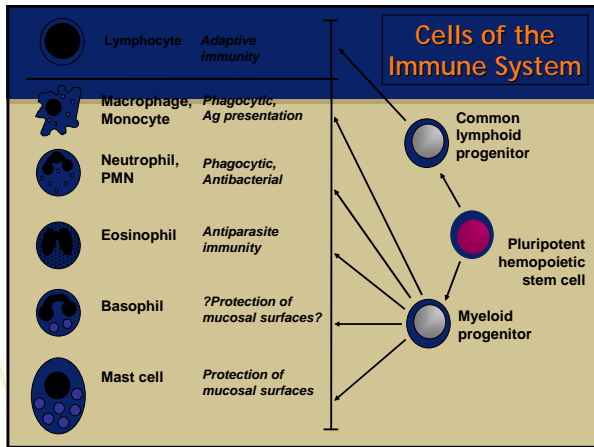
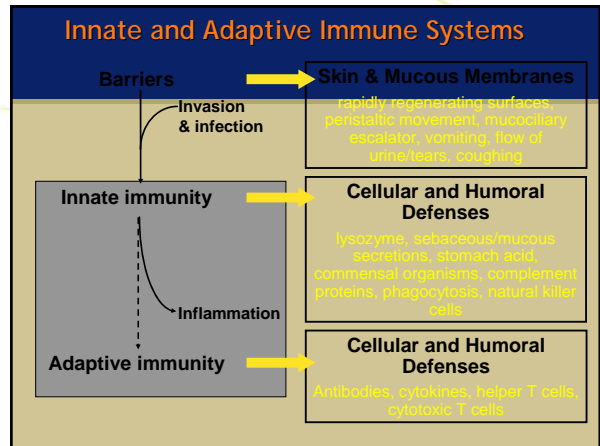


Targeting Cancer-Specific Mutations with Immunotherapy

Donald Bellgrau
University of Colorado



Role of the Immune System and Cancer

Ilya Mechnikov
 Paul Ehrlich
 Nobel Prize 1909

Immune Surveillance Escape of Cancer
 All people develop tumors, but the immune system normally kills them

<http://nobelprize.org/medicine/laureates/1908/>

Immunotherapy

Waking up the immune system

Immunology 101

Is it self?

Self non self discrimination is not enough

Is it dangerous?

How do we know it is dangerous?

Answer: The expression of pathogen associated molecular patterns (PAMPs)

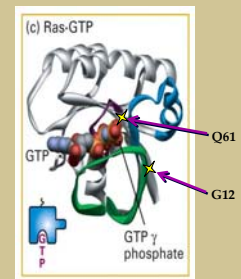
Why do tumors evade the immune response?

Answer: They evolve from us so the immune system thinks they are *self* and they do not express pathogen associated molecular patterns so even if they do not look like self they are perceived as *not dangerous*.

Finding a non self target on cancer cells

Ras is the most commonly mutated oncogene in cancer

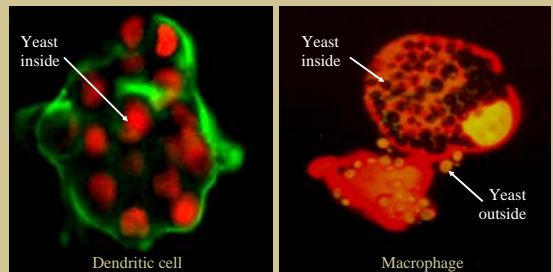
- Pancreatic > 90%
- Colorectal - 35%
- NSCLC - 25%



Making the mutated ras look dangerous

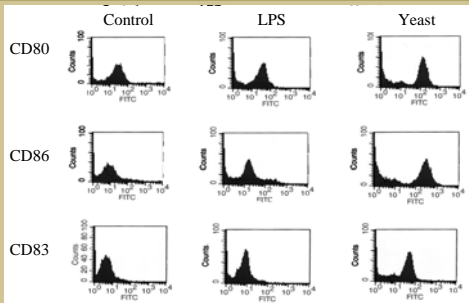
Solution: Present it with PAMPs to the immune system.

Uptake of Yeast by antigen presenting cells



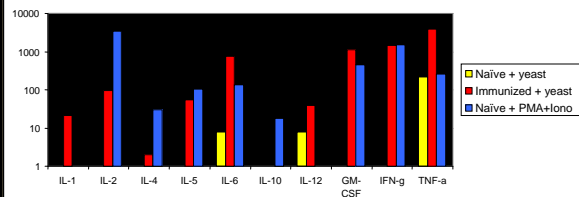
Stubbs et al. (2001) *Nature Medicine* 7, 625-629.
 "Whole recombinant yeast vaccine activates dendritic cells and elicits protective cell-mediated immunity."

Human dendritic cell maturation



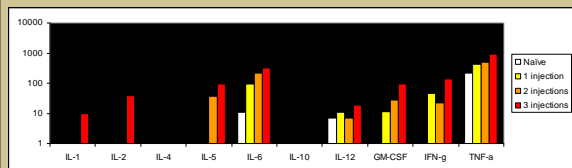
in collaboration with Dr. Virginia Borges

Whole yeast induce Th1 cytokines



BALB/c T cells (immunized 3X) - IVS w/ GI-5005

Cytokine production increases as immunization increase



BALB/c T cells - IVS w/ GI-5005

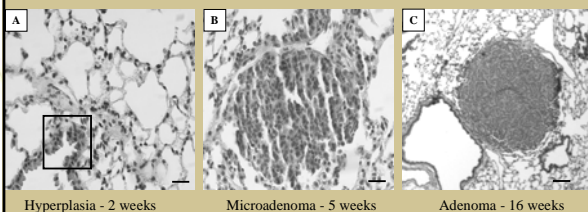
The whole yeast based concept

Heat-killed recombinant *S. cerevisiae* transfected with gene(s) for target antigen



- Multiple antigens
- Defined protein content
- Dosing: 1 YU = 10⁷ Cells

Urethane induced tumors

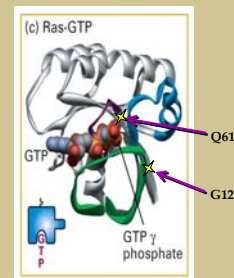


Scale bar: 10 μm panels A and B; 50 μm panel C
Figure provided by Drs. Kistley, Nield and Malkinson

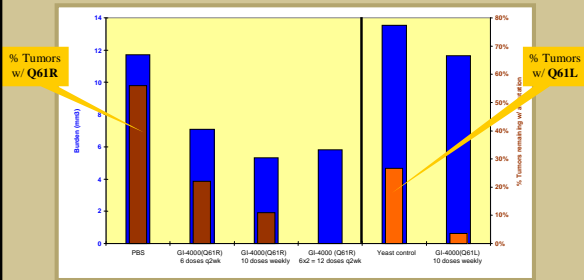
Finding a non self target on cancer cells

Ras is the most commonly mutated oncogene in cancer

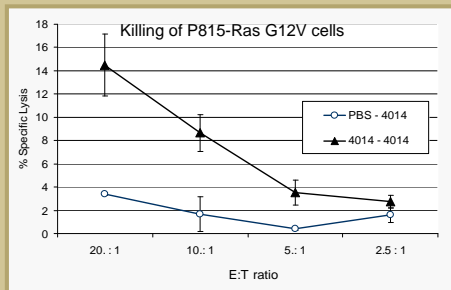
- Pancreatic > 90%
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Mutation-specific tumor ablation



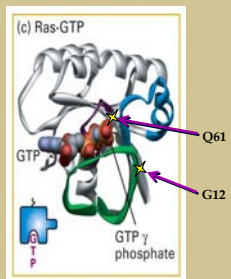
Mutant ras-specific cytotoxic T cells



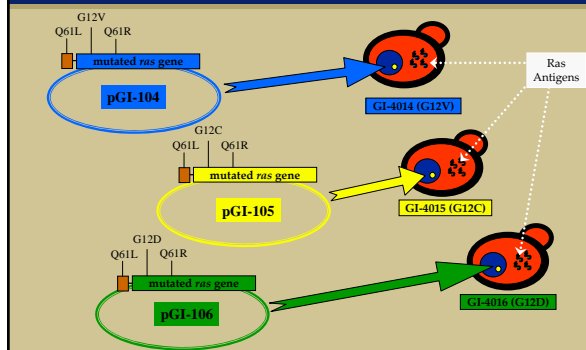
Finding a non self target on cancer cells

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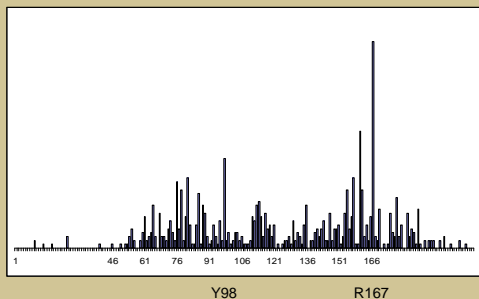
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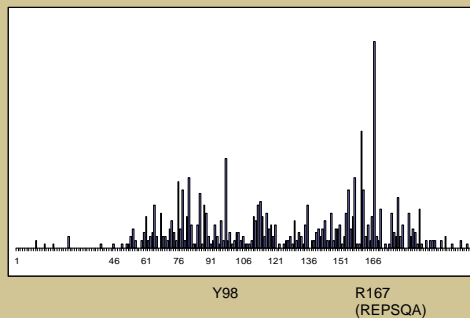
GI-4000 targets mutated Ras



VHL mutations



VHL mutations



Problem!!!

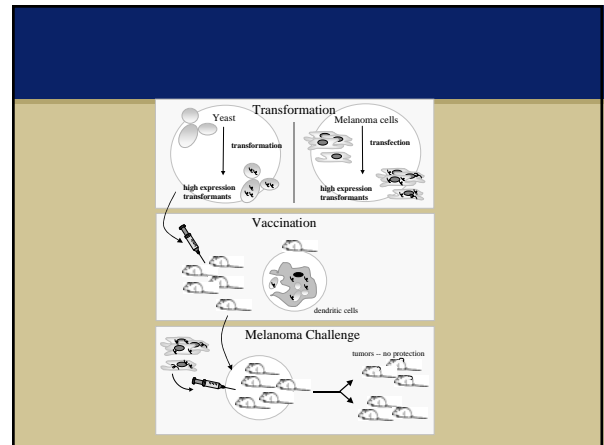
No mouse model for VHL renal cell carcinoma

VHL species specificity

- 58 Tyrosine98 117
hVHL-RPRPVLRSVNSREPSQVIFCNRSRVLPLVWLNFDGEPQYPYTLPPGTGRRHSYRGHLW
- mVHL-RPRPVLRSVNSREPSQVIFCNRSRVLPLVWLNFDGEPQYPYTLPPGTGRRHSYRGHLW
- 118 Arginine167 177
hVHL-LFRDAGTHDGLLVNQTELVPSLVNDGQPIFANTLPPVYTLKERCLQVVRSLVKPENYRR
- mVHL-LFRDAGTHDGLLVNQTELVPSLVNDGQPIFANTLPPVYTLKERCLQVVRSLVKPENYRR
- 178 211
hVHL-LDIVRSLYEDLEHPNVQKDLERLTQERIAHQRM
- mVHL-LDIVRSLYEDLEYPVSRKDIQRLSQEHLESQHL

Preclinical proof of concept targets

- Y98 is most frequently mutated into histidine, while R167 is typically mutated to glutamine or tryptophane.
- R167 is also affected by frame shift mutations; an insertion of a single G residue within the R167 codon will generate a novel frame shifted peptide (REPSQA) followed by a STOP codon (TGA).
- Thus we generated both a histidine missense mutation at Y98 (Y98H) and a frameshift mutation at R167 (R167fr) to create potentially immunogenic mutant VHL proteins that recapitulate features of known human VHL mutations.



In vivo efficacy

Mouse #	Immunization	Tumor Challenge	Tumor Growth
9	none	B16 untransfected (3) B16 VHL WT (3) B16 VHL ΔMut (3)	3/3 3/3 2/3
18	VHL WT yeast	B16 untransfected (6) B16 VHL WT (6) B16 VHL ΔMut (6)	5/6 5/6 3/6
18	VHL ΔMut yeast	B16 untransfected (6) B16 VHL WT (6) B16 VHL ΔMut (6)	5/6 4/5 (one died) 0/6

Mouse #	Immunization	Tumor Challenge	Tumor Growth
6	VHL ΔMut yeast	B16 untransfected	5/6 mice developed tumors. One mouse sacrificed early due to complications.
6	VHL ΔMut yeast	B16 VHL WT	5/6 mice developed tumors.
6	VHL ΔMut yeast	B16 VHL ΔMut	0/6 mice developed tumors.

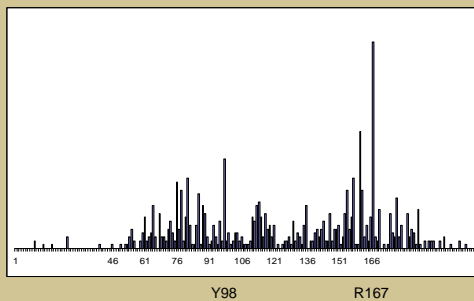
Rationale for VHLFA Grant

- In collaboration with the Cleveland Clinic, we and our collaborators (Drabkin/Gemmill group) typed tumors from 28 RCC patients. These analyses continue. **Thus far no two patients have the same mutation**
- however 11 have identified mutations potentially useful to vaccine development. The identified mutations are 1) a 9 bp deletion the deletes PQYP at 95-98 and adds H before termination, 2) S80R, 3) a 2bp mutation at DG 143-44 leading to a D stop, 4) a frame shift at codon 142, 5) a deletion of QTG at 132-134 leading to an H stop 6) a one bp deletion YE at 185-186 leading to a Y stop, 7) a frame shift deletion KE 159-160 leading to a KS with a stop at 168 8) a P25L, 9) a R200W, 10) a N78S and 11) a C162Y.

Question: Are all mutations targets for the whole yeast based immunotherapy

- **If so, do different mutations contained within the same yeast continue to be targets (i.e. is there antigenic competition)?**
- *If so, can we make one yeast product that covers some, more common mutations shared by a cohort of potential patients?*
- *If not, can we convince FDA to treat all VHL mutation-expressing yeast as the same.*

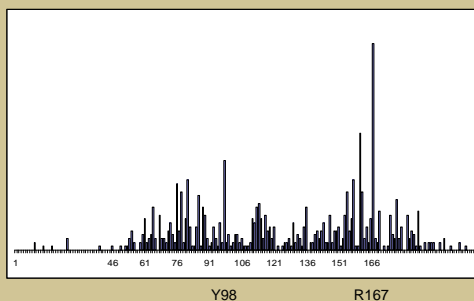
VHL mutations



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VHL mutations



Where we are in our first year of funding

- The mutant version of VHL begins at the same internal methionine residue and contains a point mutation at amino acid 100 (T100V) and is truncated at amino acid R167.
- These two constructs have been ligated into a yeast-based vector (pYEX-BX) with a copper inducible promoter and are currently being ligated into a mammalian vector (pBK-RSV).

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Tarmogen manufacturing: Wave Bioreactor

- Current in-house scale
 - 50 liter bags = 25 Liter runs
 - 3000 - 4000 YU / liter / run
 - 75,000 - 100,000 YU / run
- Scalable technology
- Wave Bags up to 1000 liters
 - 500 liters of product
 - 100,000 doses / run / reactor
 - 1 run = 1 week



Wave Bioreactor

Whole yeast based immunotherapy

- Produce precise amount of target protein
- Multiple antigen delivery
- Durable T cell-mediated immune responses
- Dose response by
 - Number of yeast
 - Antigen content
 - Number of administrations
- Not neutralized with repeated administration
- Non-toxic in multiple animal species / humans
- Simple manufacturing

A history of immunology leading to immunotherapy

