Biomarker development for immunotherapy using peripheral blood

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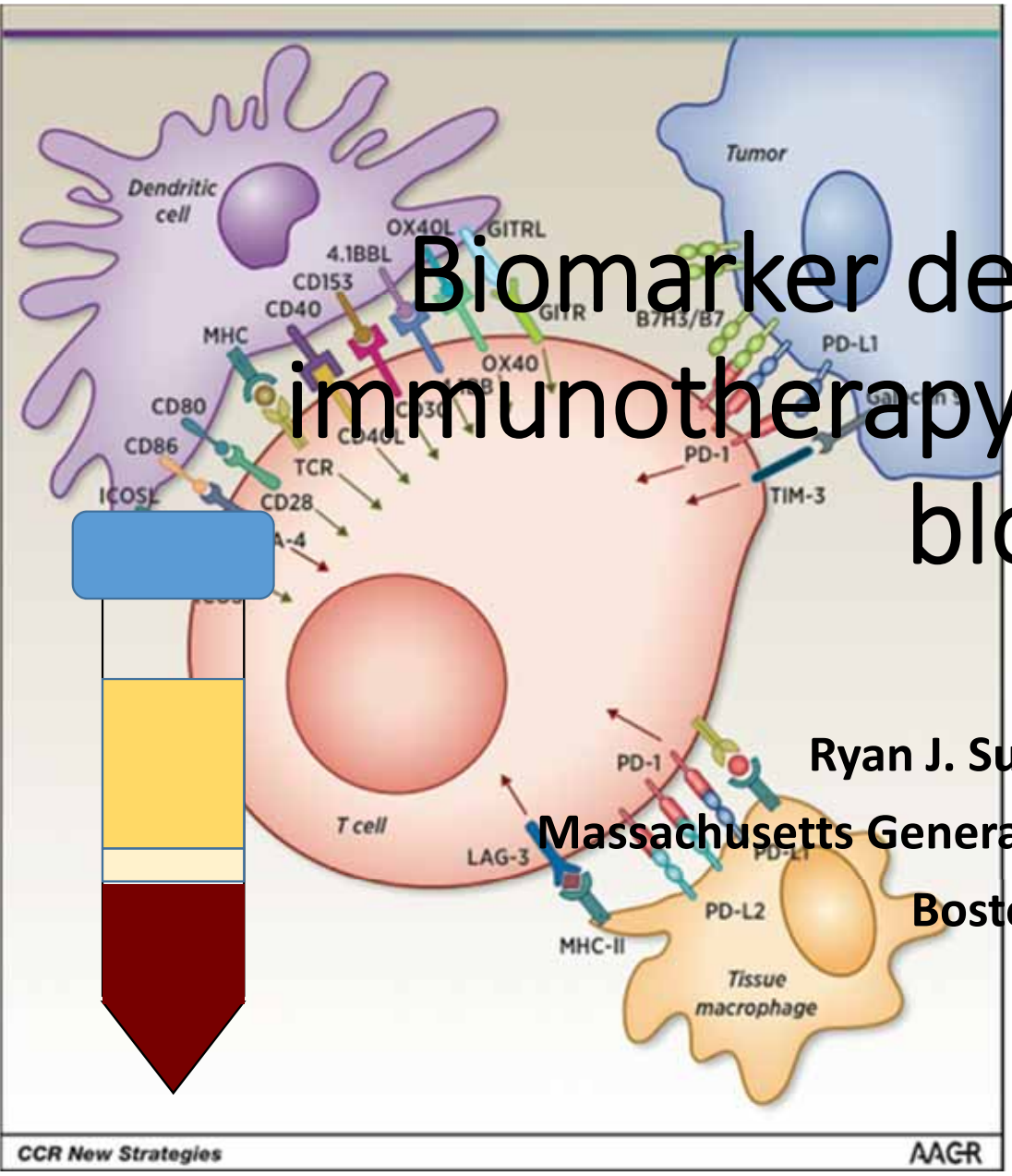
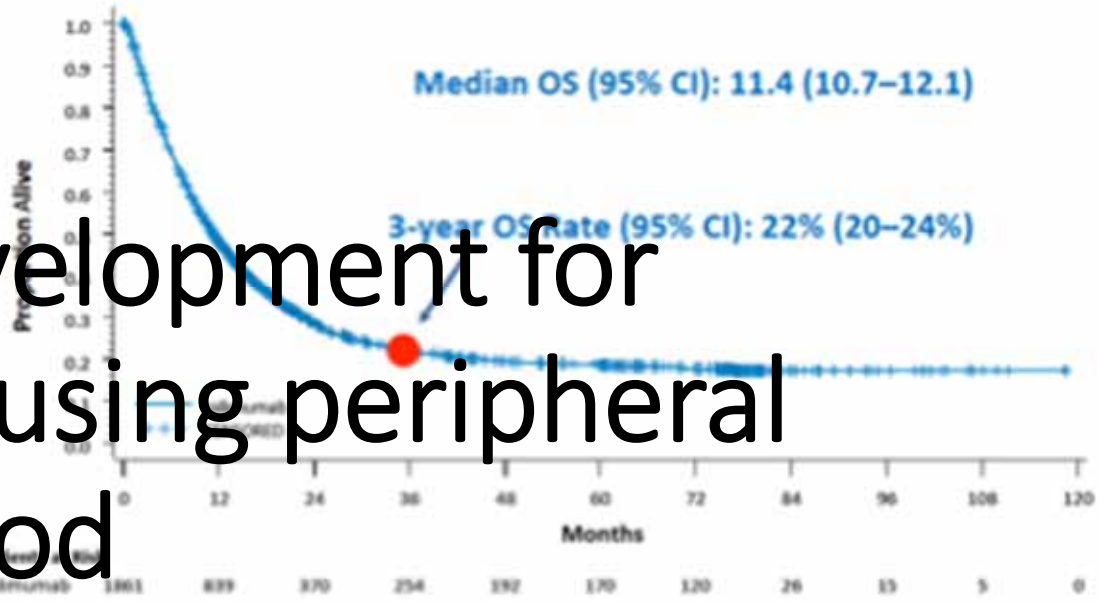
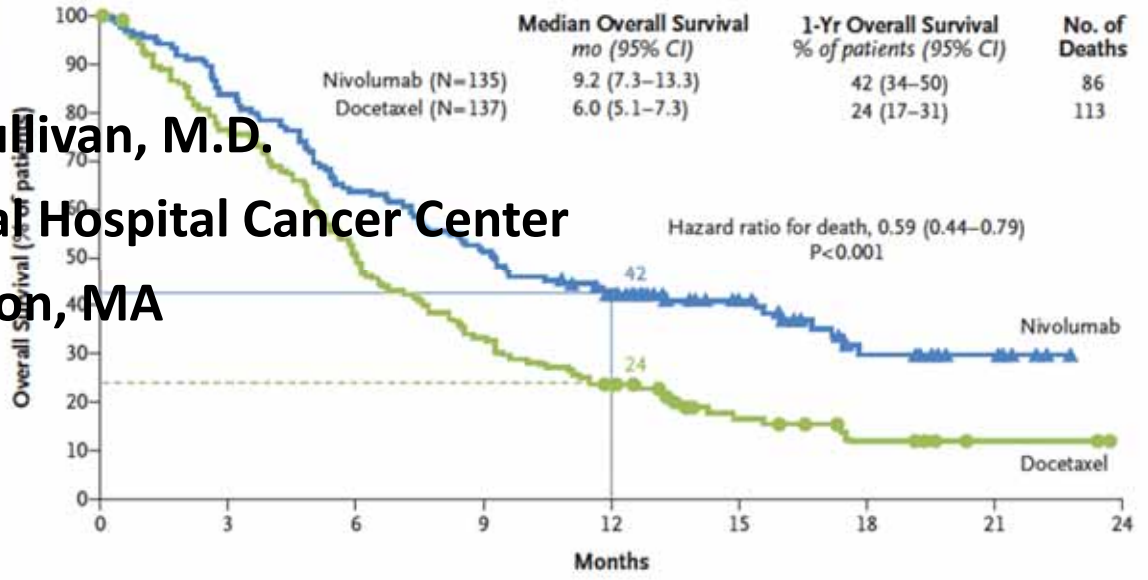






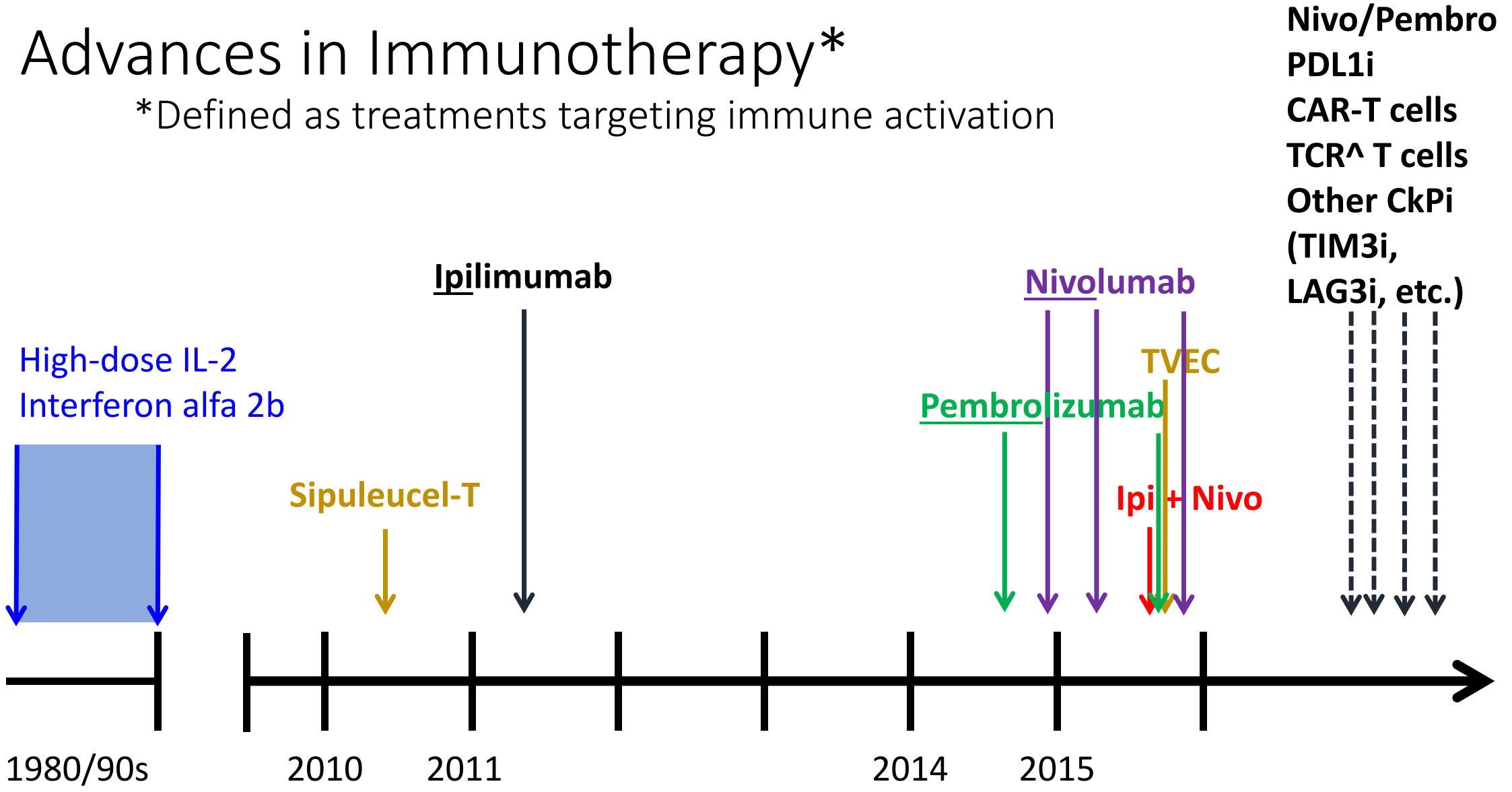
Disclosures

- Advisory Board/Consulting:
  - Novartis
  - Biodesix
  - Prometheus

- Research Sponsorship:
  - Biodesix
  - Exosome Diagnostics
  - Adaptive Biotechnologies
  - Merck
  - Bristol Myers Squibb
  - Prometheus

Advances in Immunotherapy*

*Defined as treatments targeting immune activation



1. Who is going to benefit from immunotherapy?

2. Will we able to detect time and mechanism of resistance to immune therapy?

Blood-based biomarker development: Blood vs Tissue

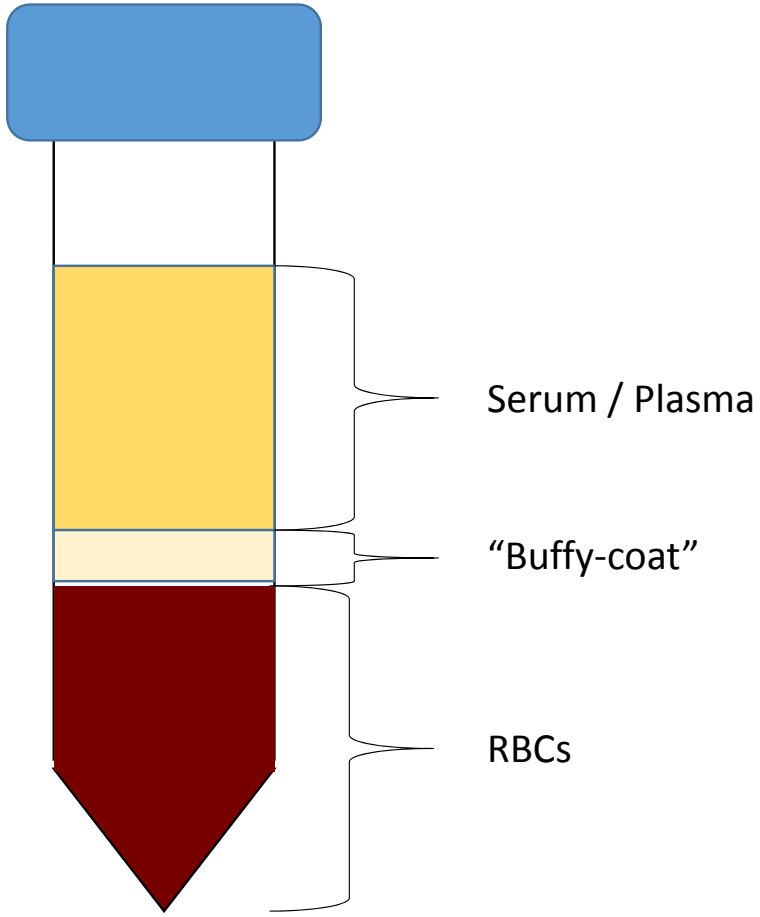
Advantages of blood analysis

- Accessibility / Safety
- Serial sampling is much easier
- Blood may be reflective of entire disease burden (heterogeneity)
- Amenable to analysis to virtually every platform of testing (flow cytometry, ELISA, Mass spectometry, nucleic acid sequencing, etc.)
- Ready access to normal samples for comparative analysis

Advantages of tissue analysis

- Gold standard
- Sample is enriched for tumor
  - As opposed to blood which has other shed elements competing with tumor signal
  - More amenable to nucleic acid sequencing (WES/ESG, RNA sequencing)
- The tumor microenvironment is present and evaluable for physical interaction (IHC, IF, etc.)

Blood-based biomarker development

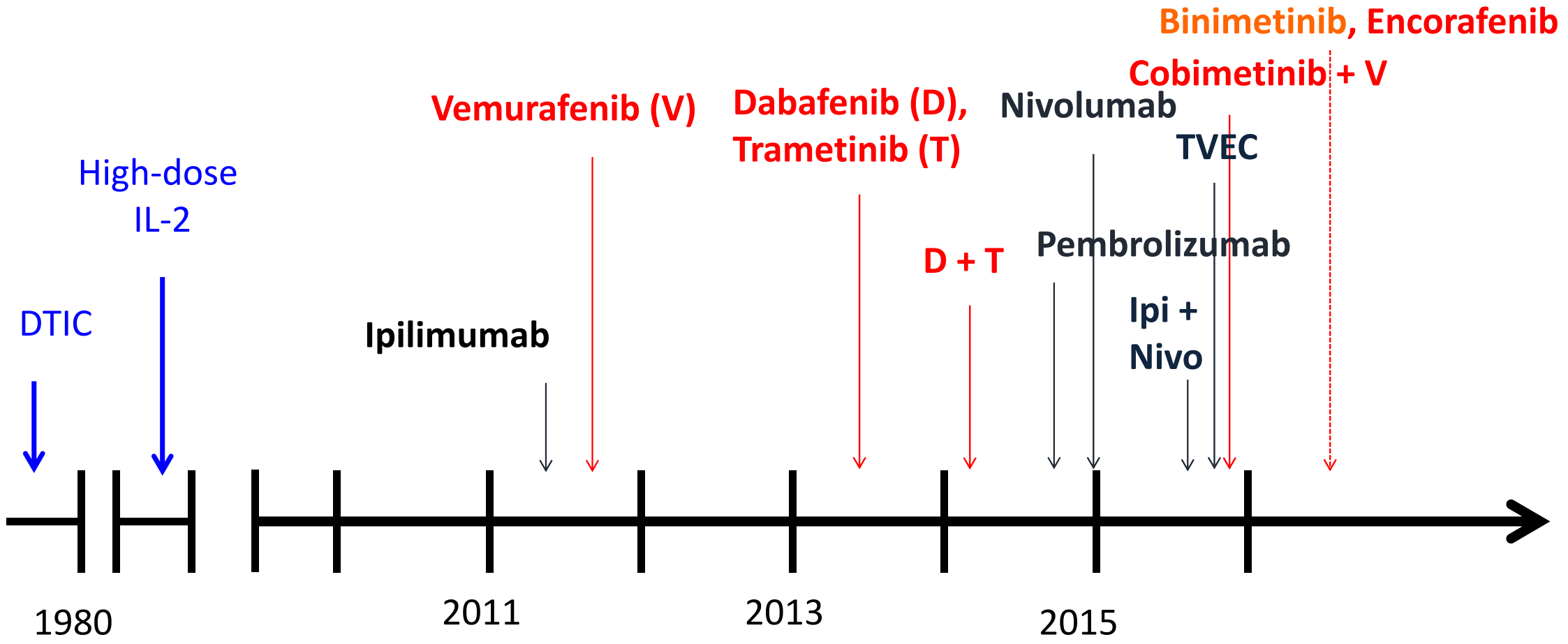


- Serum / Plasma
  - Proteins
  - Exosomes
  - cfDNA
- Buffy Coat
  - PBLs
  - Other immune cells
  - CTCs
  - Platelets
- RBCs

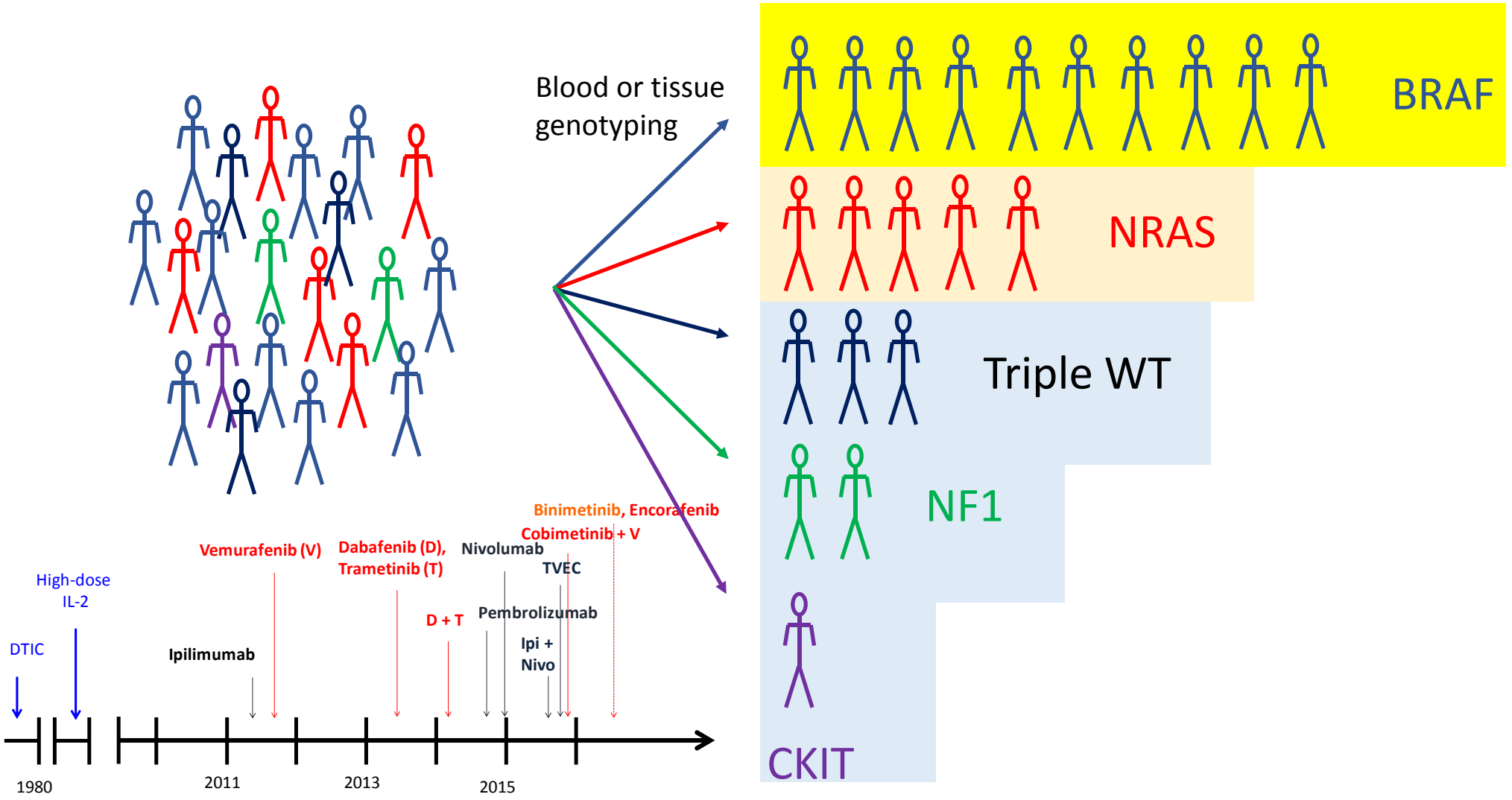
1. Who is going to benefit from immunotherapy?

USING BLOOD BASED ASSAYS TO OPTIMIZE SELECTION STRATEGIES

Optimizing Selection Strategy: Melanoma Model



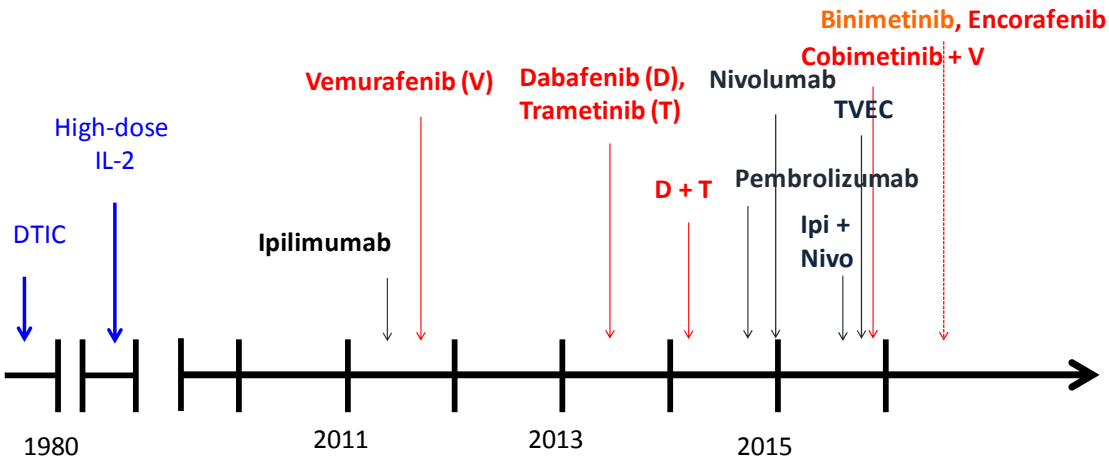
Optimizing Selection Strategy: Melanoma Model #1

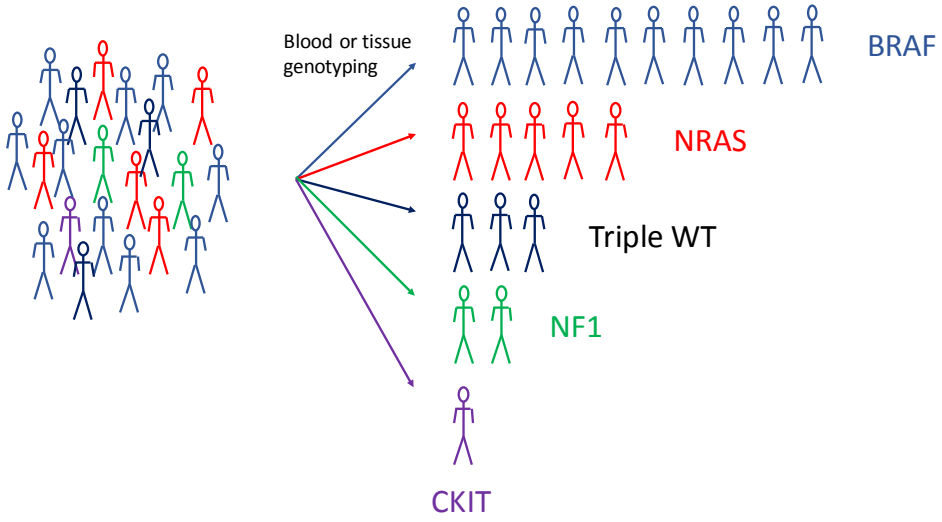


Optimizing Selection Strategy: Melanoma Model #1

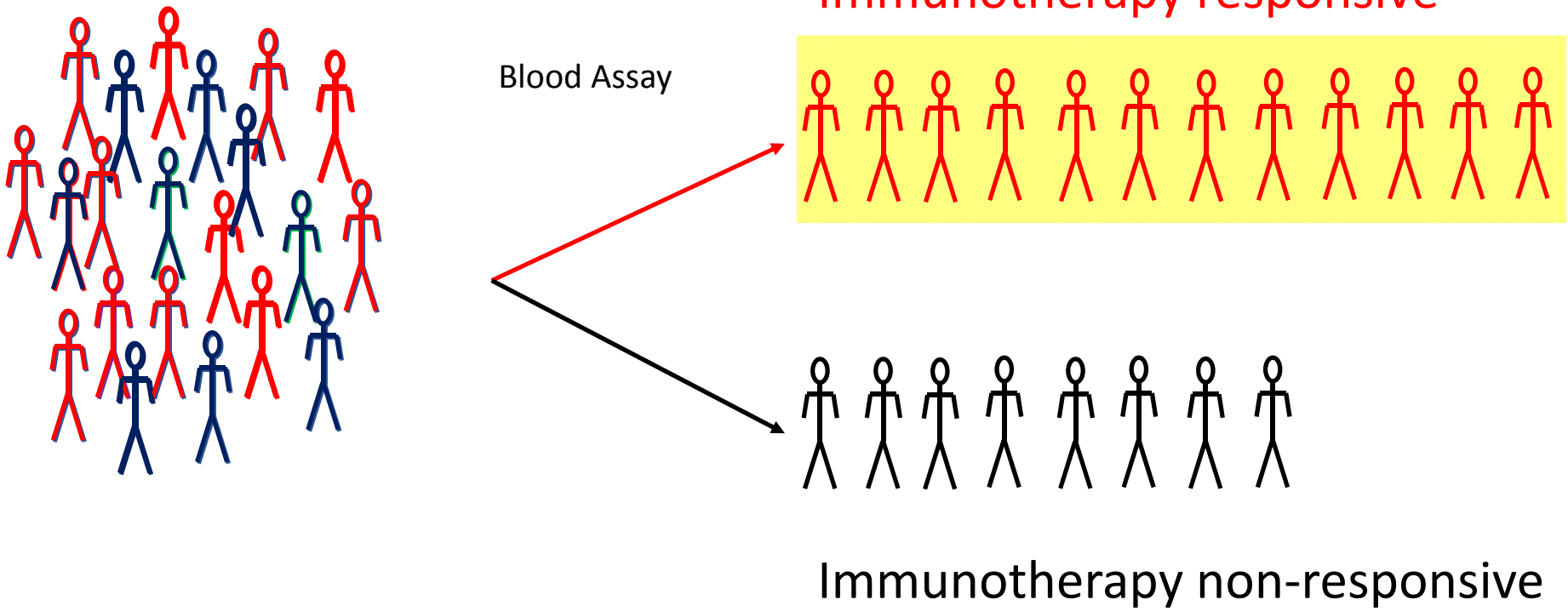
- Entirely dependent on:
  - Genotyping (blood or tissue)
  - Availability of targeted therapies for specific genotypes

- Selection of immunotherapy by default or gestalt



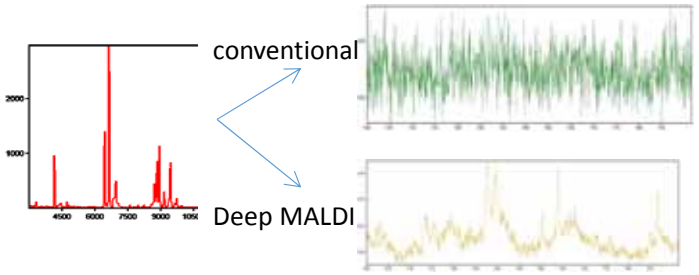


Optimizing Selection Strategy: Melanoma Model #2



Use of serum profile to predict outcome to anti-PD1 antibody therapy in melanoma (Biodesix)

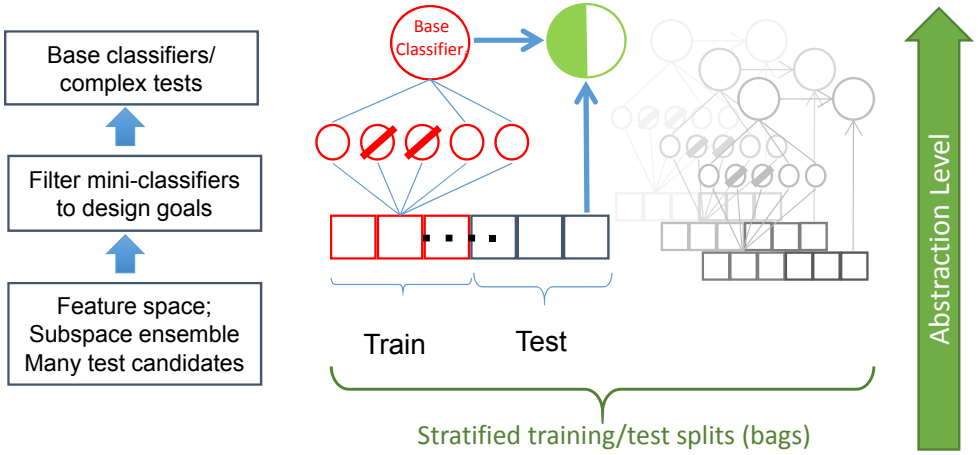
Reproducible, high throughput protein expression measurement with deep MALDI



- Measure expression of protein fragments/peptides
- Median CV < 10%
- 4-4.5 orders of magnitude dynamic range

Design clinically useful tests from spectral and clinical data using methods adapted from deep learning

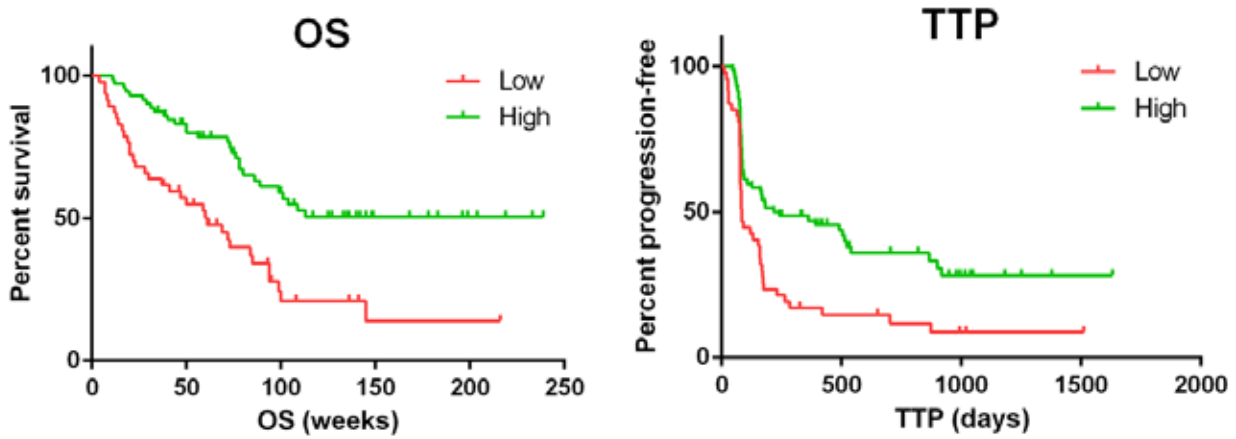
- Uses hierarchical approach with increasing levels of data abstraction
- Create tests to stratify patients by outcome (overall survival)
- Get reliable performance estimates from development set by 'out-of-bag' estimates
- Validate tests on independent sample sets



Weber et al. SITC 2015

Use of serum profile to predict outcome to anti-PD1 antibody therapy in melanoma (Biodesix)

- Development (J. Weber)
  - Pre-treatment serum samples from 119 patients with advanced melanoma in clinical trial of nivolumab with or without peptide vaccine (NCT01176461)
    - At least one prior therapy
    - 74% ipilimumab-refractory
    - PS 0-1
  - 72 (61%) patients in BDX008 high
  - 47 (39%) patients in BDX008 low

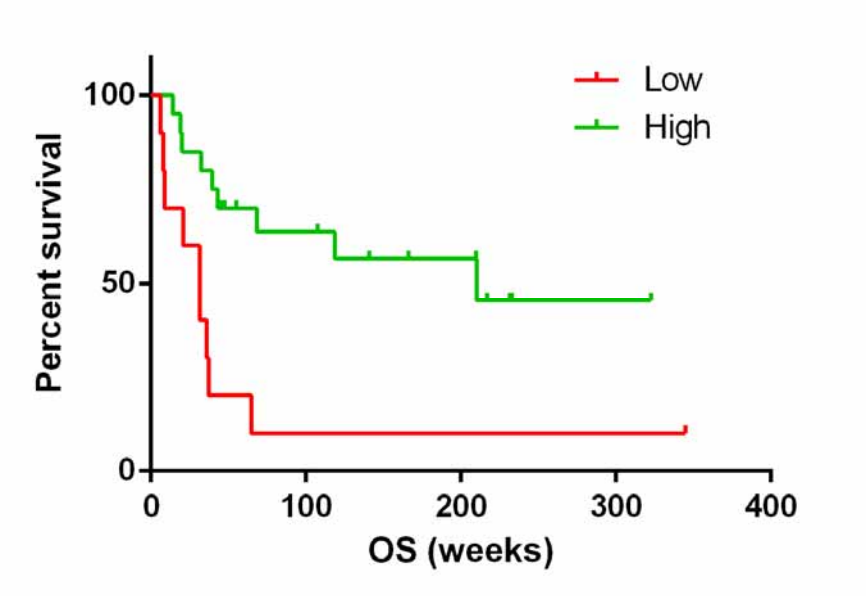


	OS	TTP
HR (95% CI)	0.38 (0.19-0.55)	0.50 (0.29-0.71)
log-rank p	<0.001	0.001
Median BDX008 low | BDX008 high	61 weeks | Not reached	84 days | 230 days

Weber et al. SITC 2015

Use of serum profile to predict outcome to anti-PD1 antibody therapy in melanoma (Biodesix)

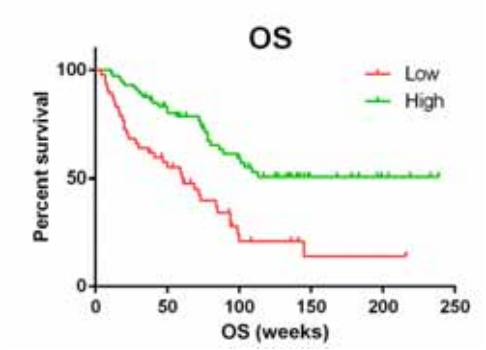
- Independent Validation (M. Sznol, H. Kluger, R. Halaban)
  - Pre-treatment serum samples from 30 patients with advanced melanoma treated with anti-PD1 therapy at Yale
    - Observational
  - 20 patients in BDX008 high
  - 10 patients in BDX008 low

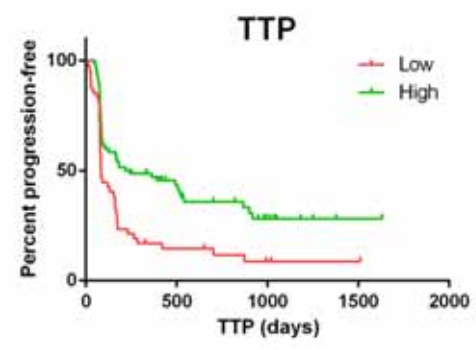


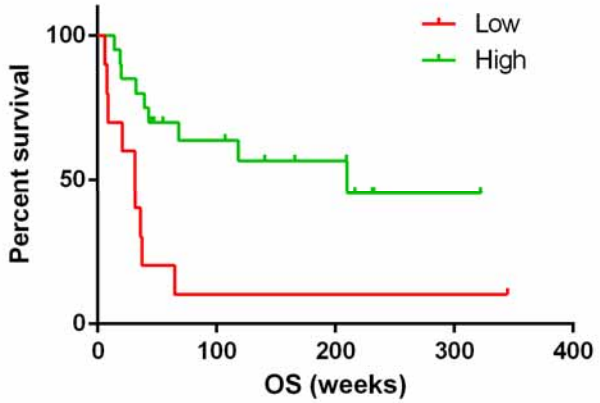
	OS
HR (95% CI)	0.27 (0.05-0.52)
log-rank p	0.002
Median BDX008 low	32 weeks
Median BDX008 high	210 weeks

Weber et al. SITC 2015

Use of serum profile to predict outcome to anti-PD1 antibody therapy in melanoma (Biodesix)



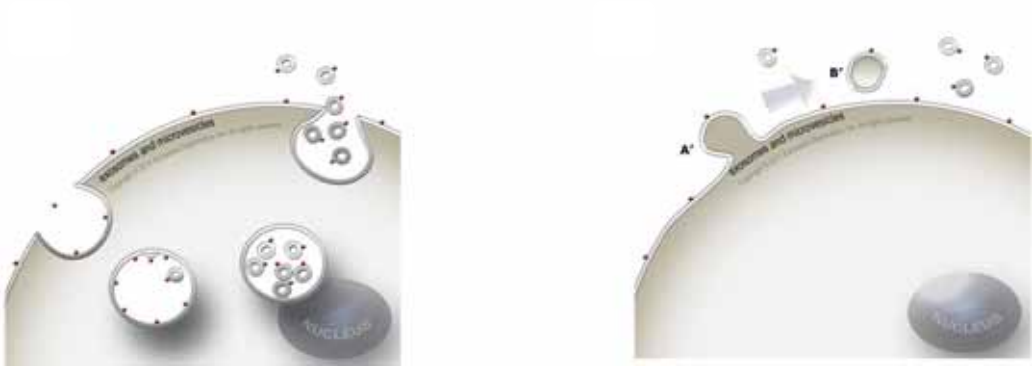


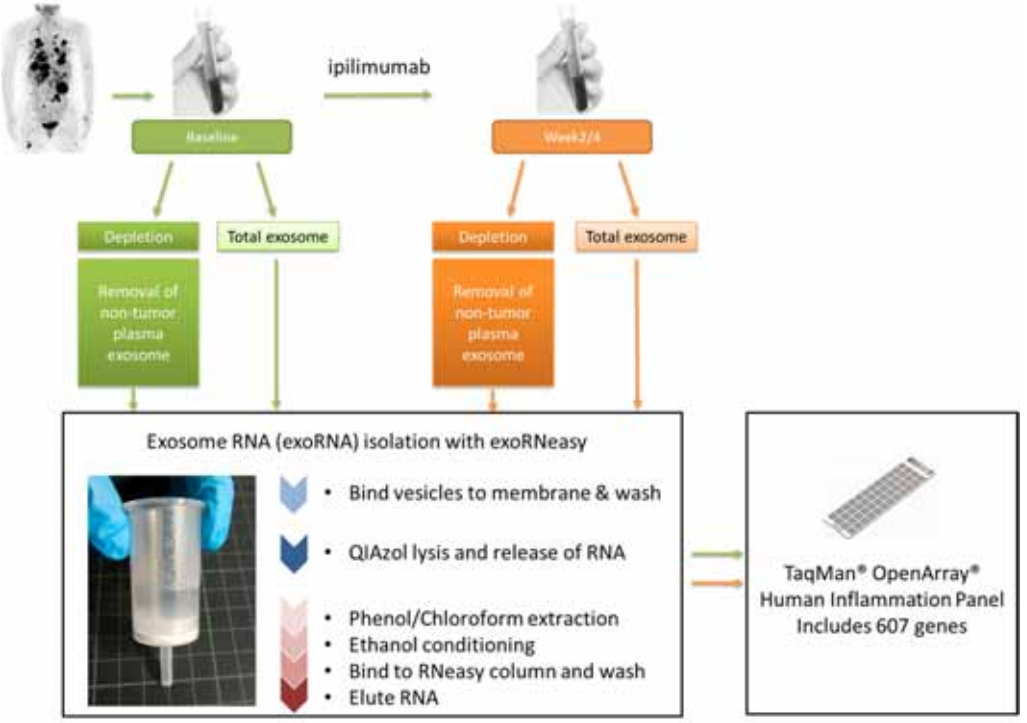


Weber et al. SITC 2015

- BDX008 is a protein signature developed in an unbiased manner using deep learning
- Protein levels are detected using MALDI, offering excellent discrimination of specific proteins
- "Gene" set enrichment analysis shows the following when comparing BDX008 high vs low
  - Acute inflammatory response ($p<0.02$)
  - Complement system ($p=0.01$)
  - Acute phase reactants ($p=0.01$)
- Further validation is ongoing

Use of plasma exosome profile to predict outcome to ipilimumab in melanoma (Exosome Diagnostics)

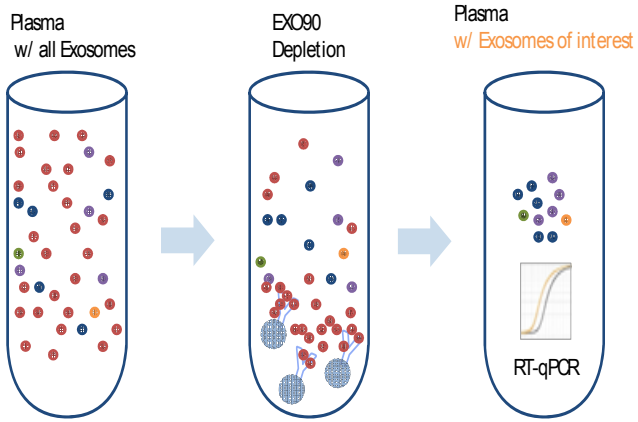


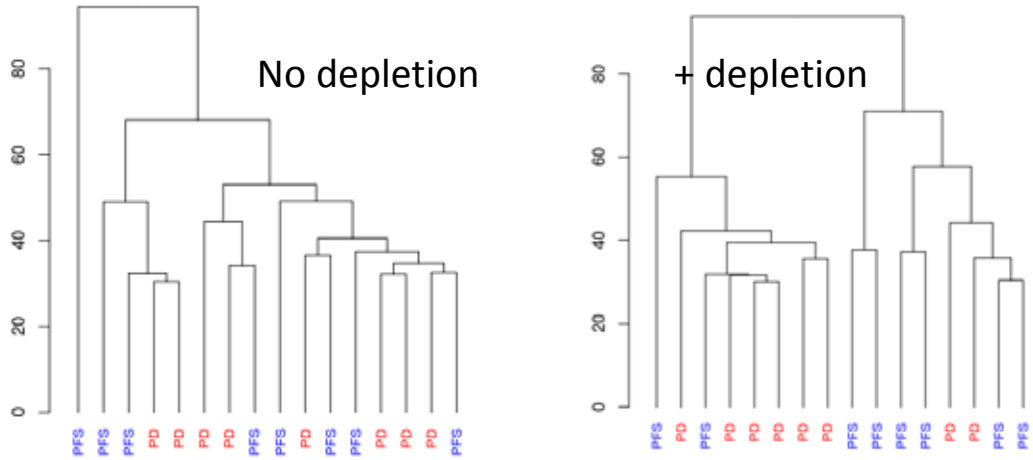


Coticchia et al. Mol Targets Cancer Ther. 2015

Use of plasma exosome profile to predict outcome to ipilimumab in melanoma (Exosome Diagnostics)

Patient groups	N	Duration of PFS (mo.) Mean ± SE	Notes
Progression Free Survival (PFS) > 6 months	8	16.38 ±4.37	4 patients achieved durable response 4 patients progressed at 9.75 ± 2.39 months
Early Progressive Disease (PD)	8	2.75 ±0.49	1 patient progressed within 1 month 4 patients progressed at 2 months 3 Patient progressed between 4-5 months
Normal Human Plasma	3	NA	NA





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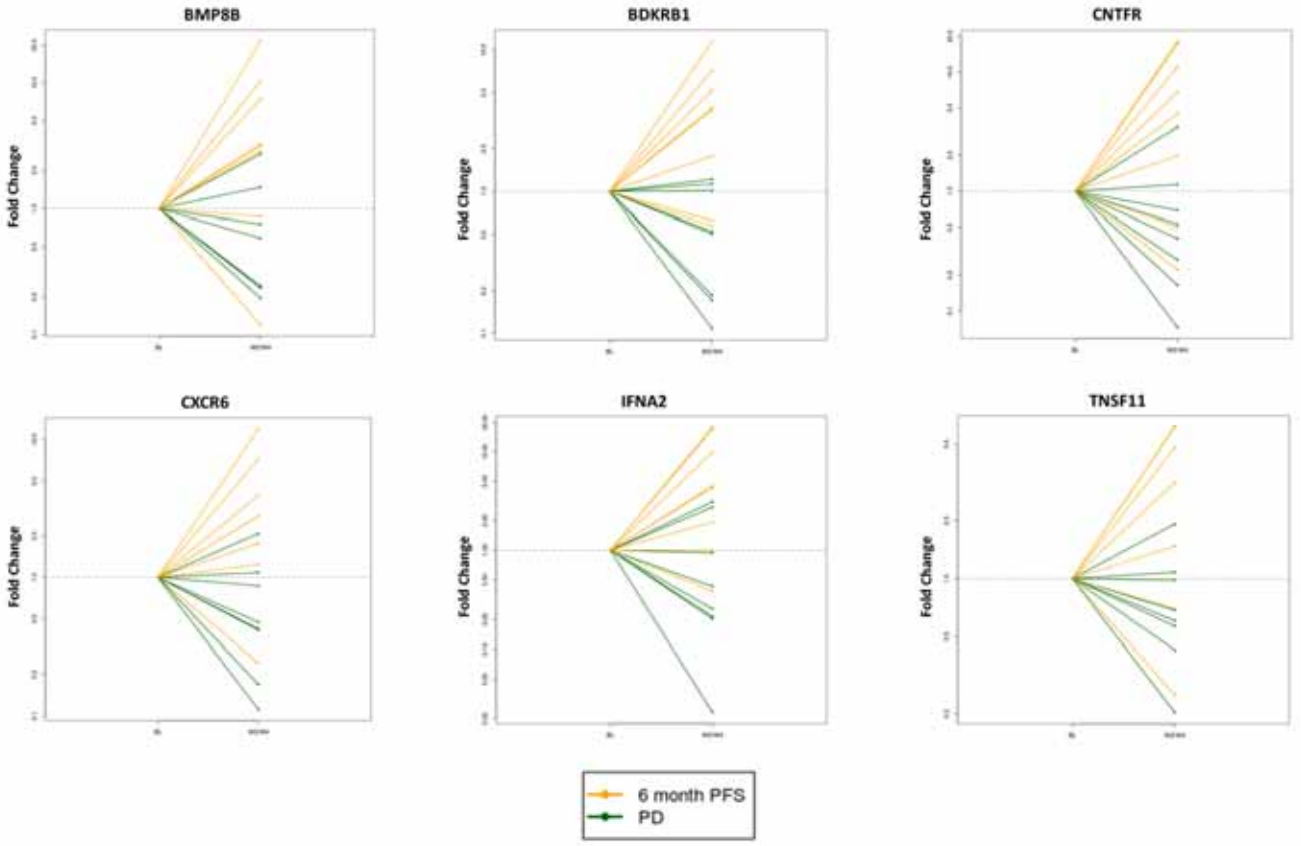
Use of plasma exosome profile to predict outcome to ipilimumab in melanoma (Exosome Diagnostics)



- Gene Selection Criteria
  - Up in >50% of PFS group

  AND
  - Down in >50% of PD group
- 10 of 607 examined genes identified using depletion method
- 0 of 607 identified in total plasma exosomes

$P < 0.0001$

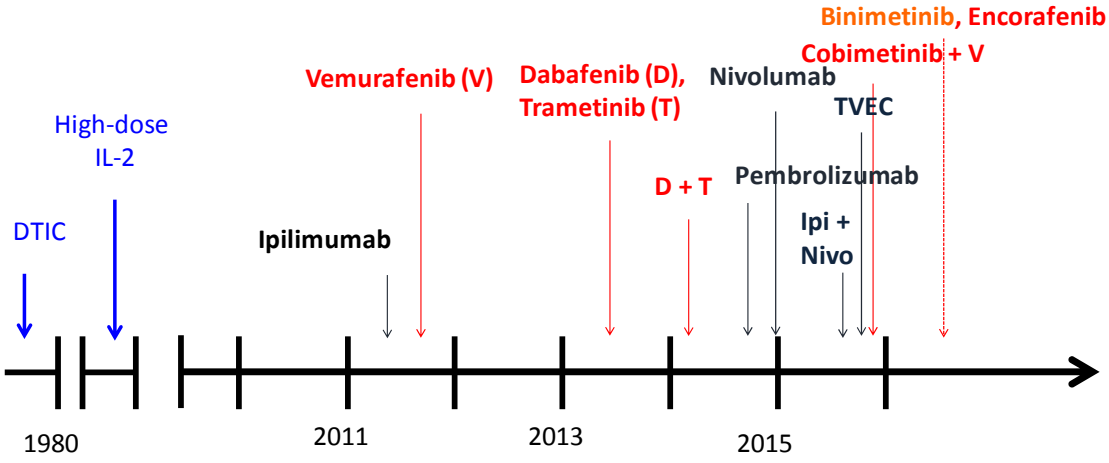
- Genes:

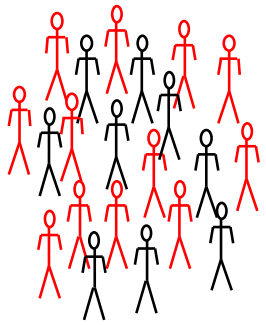
IL11, CNTFR, TNFSF11, IFNA2, IL31RA, BDKRB1, IL17B, IFNB1, BMP8B, CXCR6

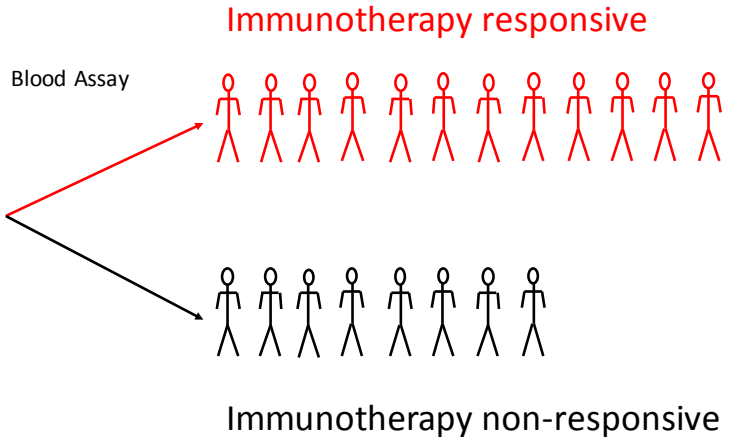
Coticchia et al. Mol Targets Cancer Ther. 2015

Optimizing Selection Strategy: Melanoma Model #2

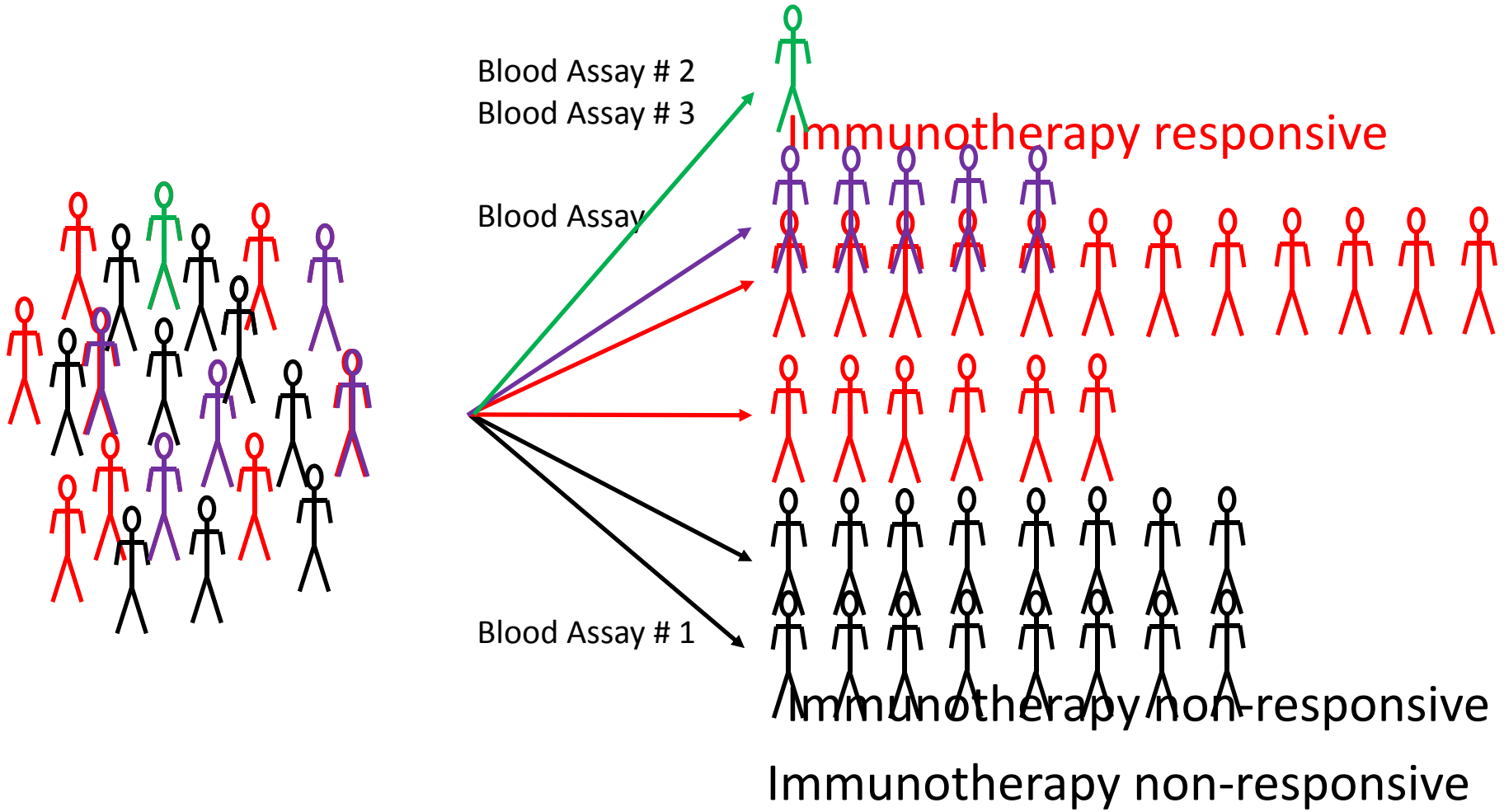
- Utilizes emerging technologies/approaches to assay blood
- As opposed to Model #1, selection of immunotherapy is active
- Does not help (yet) select amongst specific immunotherapies
- Minimizes the potential selection of long-term survivors of targeted therapy



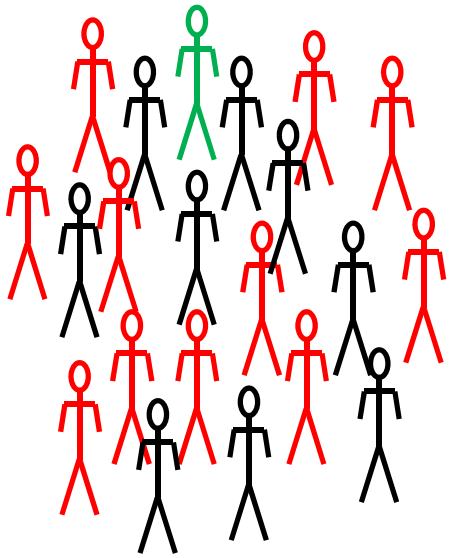




Optimizing Selection Strategy: Melanoma Model #3



Use of serum and tissue arrays to identify responders to high-dose IL2 in melanoma



Can we identify those patients who may be cured with high-dose IL2?

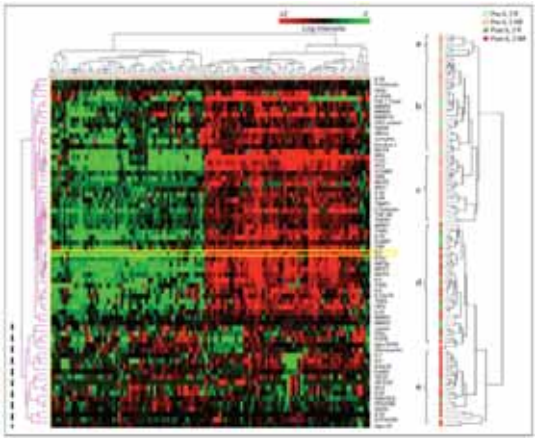
- High dose IL-2 is associated with durable benefit in ~10% of patients treated
- However:
  - It requires inpatient hospitalization due to severe toxicities
  - Frontline therapy potentially takes away an opportunity to receive “better” immunotherapy (anti-PD1 based treatments)
  - Very little data exists about its safety and effectiveness after anti-PD1 or anti-CTLA4 antibody therapy

Use of serum and tissue arrays to identify responders to high-dose IL2 in melanoma

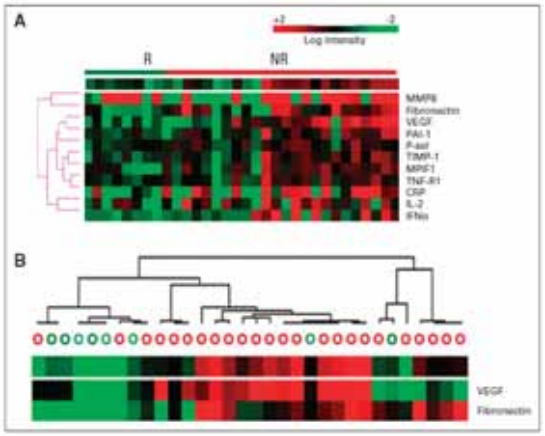
Multiplex antibody-targeted protein array platform

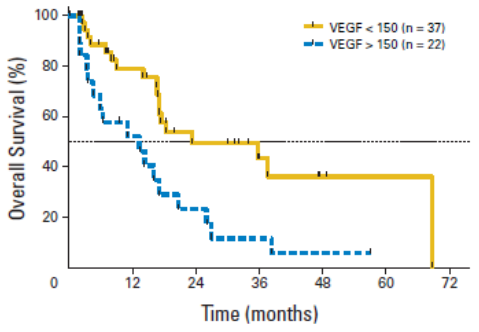
- 68 potentially relevant soluble factors were identified (test)
- 11 biomarkers associated with therapeutic outcome (validation)
- 2 were identified as independent predictors (validation)

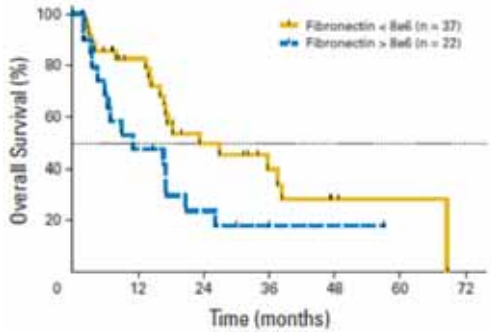
Test set



Validation set







Sabatino et al. J Clin Oncol. 2009

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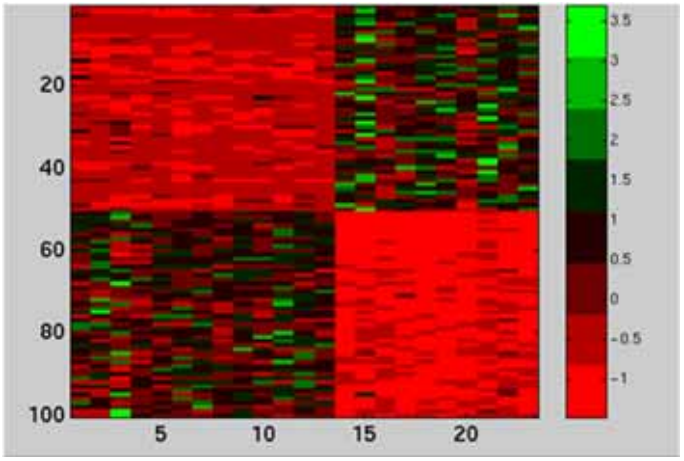


Class 1:
MITF and melanocyte antigen expression
e.g. MITF, ML-AIP, GP100, tyrosinase, MelanA

Class 2:
Immune/inflammatory genes
e.g. Annexin A1, IL6R, oncostatin M, MCSF, GMCSF, etc.

	Class 1	Class 2	p-value
Total	21	7	
Complete response	3 (14%)	2 (29%)	
Partial response	5 (24%)	4 (57%)	
Total response	8 (38%)	6 (86%)	0.077
Durable (>18 mo) response	3 (14%)	4 (57%)	0.043
Median OS	22.8	Not Reached	0.27
Median PFS	2.5	19.4	0.049

Class 2 (immune subclass)

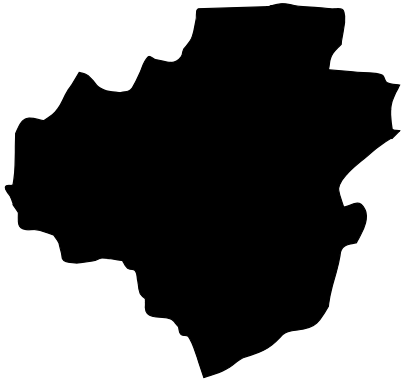
- Better PFS (p = 0.049)
- Better durable RR (p = 0.043)
- Trend towards improved RR (p = 0.077)
- OS similar

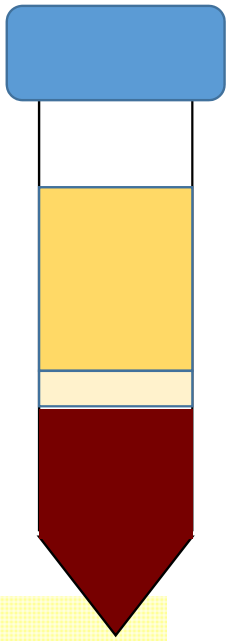
Sullivan et al. J Clin Oncol; suppl. 2009

Use of serum and tissue arrays to identify responders to high-dose IL2 in melanoma

<u>High Dose IL2 Select in Melanoma (NCT01288963)</u>

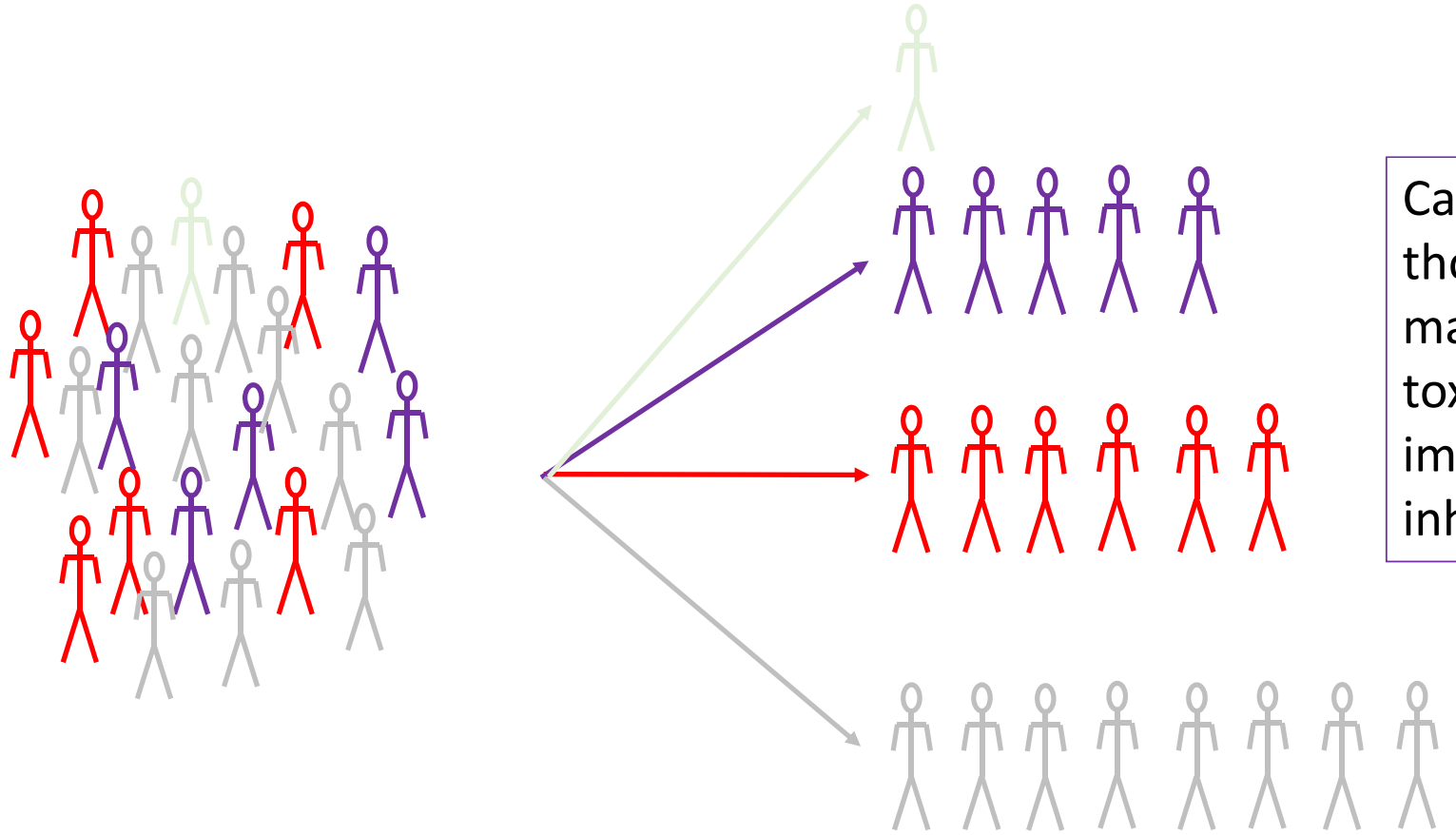
- 15 Cytokine Working Group sites
- 170 patients enrolled
- 31 PR/CR, 12 alive without PD
- ~120 with RNA/DNA available for analysis from pretreatment tumor block
- All with pretreatment isolated serum and PBMCs





- RNA sequencing (Primary endpoint)
- Serum VEGF/fibronectin (Secondary endpoint)
- Genotyping - WES (Secondary endpoint)
- Immunosequencing tumor and blood (Exploratory)
- Biodesix assay (Exploratory)
- Exosomal RNA sequencing (Exploratory)

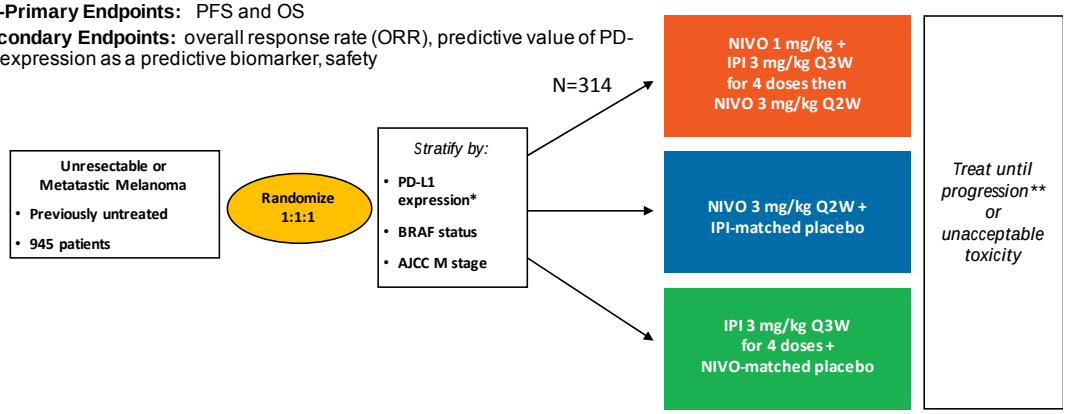
Identify responders to single-agent vs combination immune checkpoint inhibitors in melanoma



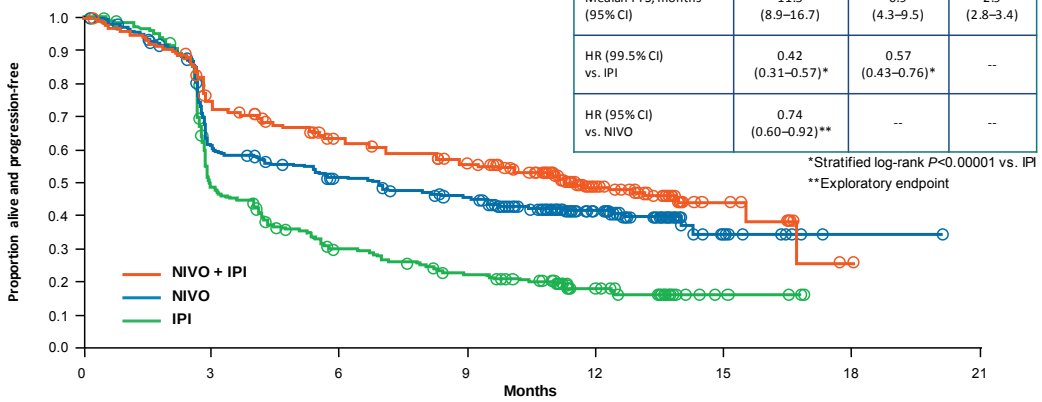
Can we identify those patients who may be be spared toxicity of combined immune checkpoint inhibitor therapy?

Identify responders to single-agent vs combination immune checkpoint inhibitors in melanoma

Co-Primary Endpoints: PFS and OS
Secondary Endpoints: overall response rate (ORR), predictive value of PD-L1 expression as a predictive biomarker, safety



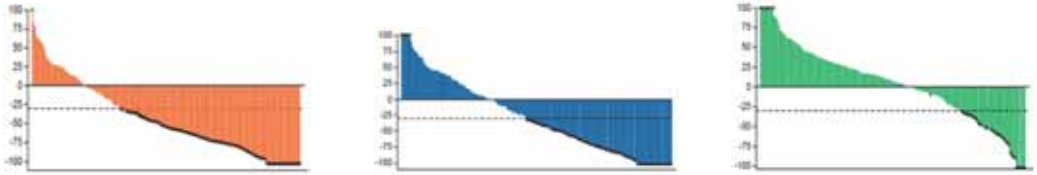
PFS (Intent-to-Treat)



	NIVO + IPI (N=314)	NIVO (N=316)	IPI (N=315)
Median PFS, months (95% CI)	11.5 (8.9–16.7)	6.9 (4.3–9.5)	2.9 (2.8–3.4)
HR (99.5% CI) vs. IPI	0.42 (0.31–0.57)*	0.57 (0.43–0.76)*	--
HR (95% CI) vs. NIVO	0.74 (0.60–0.92)**	--	--

*Stratified log-rank $P<0.00001$ vs. IPI
**Exploratory endpoint

Tumor Burden Change From Baseline



NIVO + IPI
Median change: -51.9%

NIVO
Median change: -34.5%

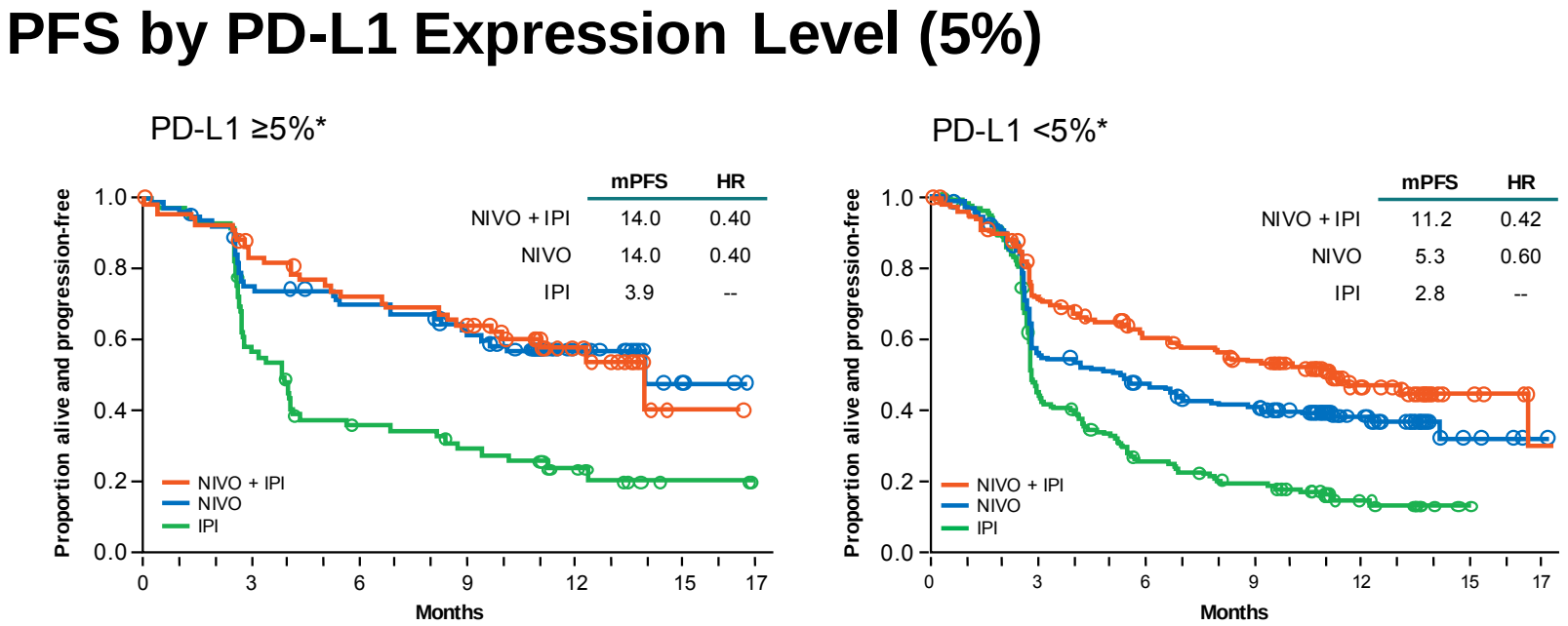
IPI
Median change: +5.9%

Patients Reporting Event, %	NIVO + IPI (N=313)		NIVO (N=313)		IPI (N=311)	
	Any Grade	Grade 3–4	Any Grade	Grade 3–4	Any Grade	Grade 3–4
Treatment-related adverse event (AE)	95.5	55.0	82.1	16.3	86.2	27.3
Treatment-related AE leading to discontinuation	36.4	29.4	7.7	5.1	14.8	13.2
Treatment-related death*	0		0.3		0.3	

*One reported in the NIVO group (neutropenia) and one in the IPI group (cardiac arrest).

Wolchok et al. ASCO 2015; Larkin et al. NEJM 2015

Identify responders to single-agent vs combination immune checkpoint inhibitors in melanoma



*Per validated PD-L1 immunohistochemical assay based on PD-L1 staining of tumor cells in a section of at least 100 evaluable tumor cells.

Wolchok et al. ASCO 2015; Larkin et al. NEJM 2015

Identify responders to single-agent vs combination immune checkpoint inhibitors in melanoma

Challenges of PDL1 testing:

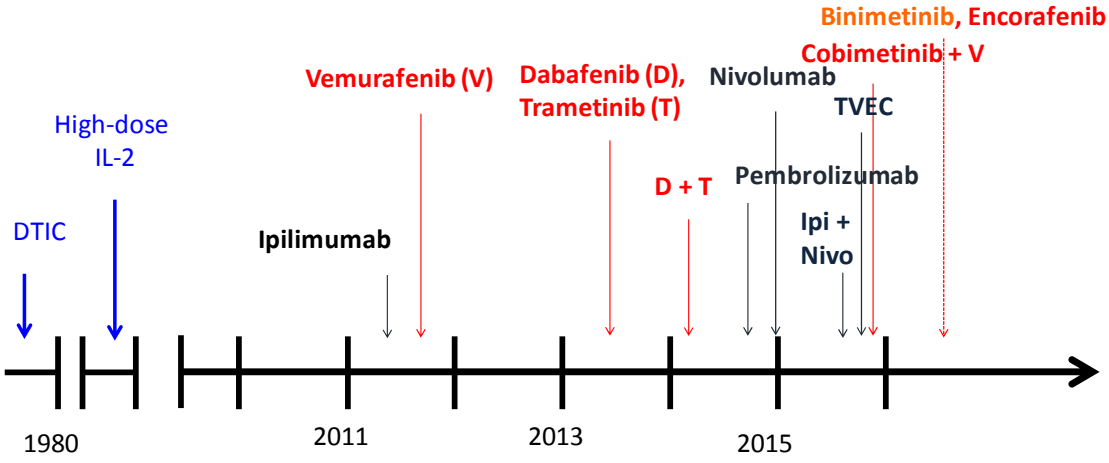
- Many assays, many targets of assay
  - e.g. Tumor vs Stromal vs Immune cell expression
- Tumor heterogeneity
- Inducible

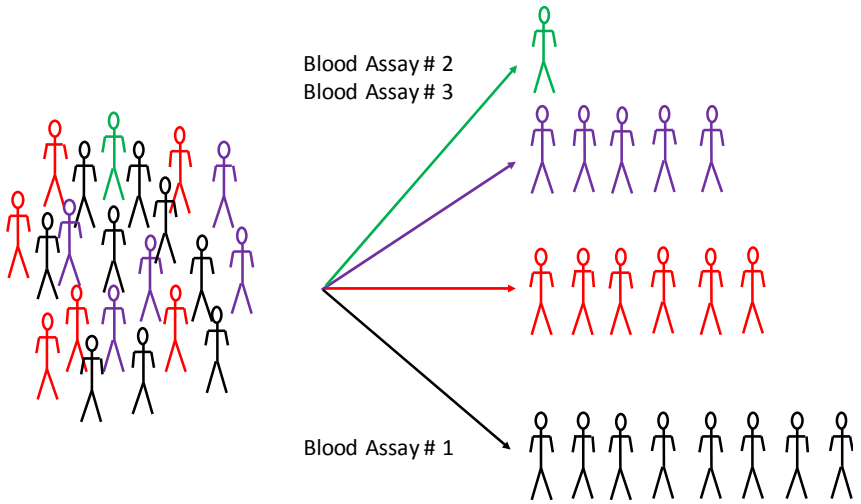
Blood PD1/PDL1 analysis theoretically gets around all these issues

- Flow for PDL1 in PBMCS by Quest (data in SLE)
- UCSF/Epic sciences (GU ASCO 2015 abstr#353; 2016 abstr#446):
  - FISH used to assess CTC PDL1 expression, no tissue expression
  - 21 patients tested, OS in 3 “hi” vs 14 “low” much improved
  - No comparison of outcomes with PD1/PDL1 inhibitors
- UCLA (Di Carlo) 2015 AACR#1582/Triple meeting abstr B98
  - Vortex HC chip (microfluidics)
  - Compared to tumor testing, correlated with response to PD1i in NSCLC

Optimizing Selection Strategy: Melanoma Model #3

- Utilizes emerging technologies/approaches to assay blood
- As opposed to Model #2, helps to select amongst specific immunotherapies
- Still minimizes the potential selection of long-term survivors of targeted therapy
- Obviously no utility in following serially to detect resistance



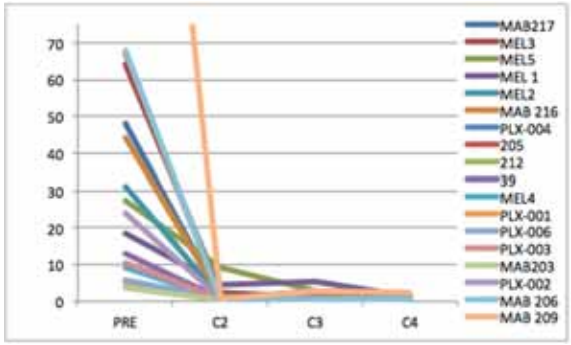


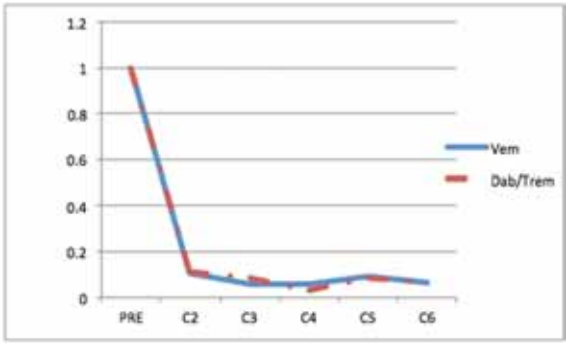
1. Who is going to benefit from immunotherapy?

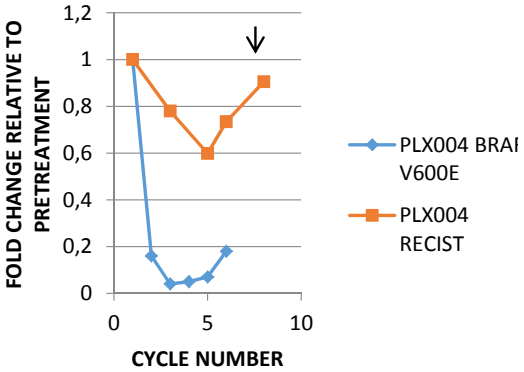
2. Will we able to detect time and mechanism of resistance to immune therapy?

USING BLOOD BASED ASSAYS TO MONITOR RESPONSE AND RESISTANCE

Response and Resistance Monitoring Strategy

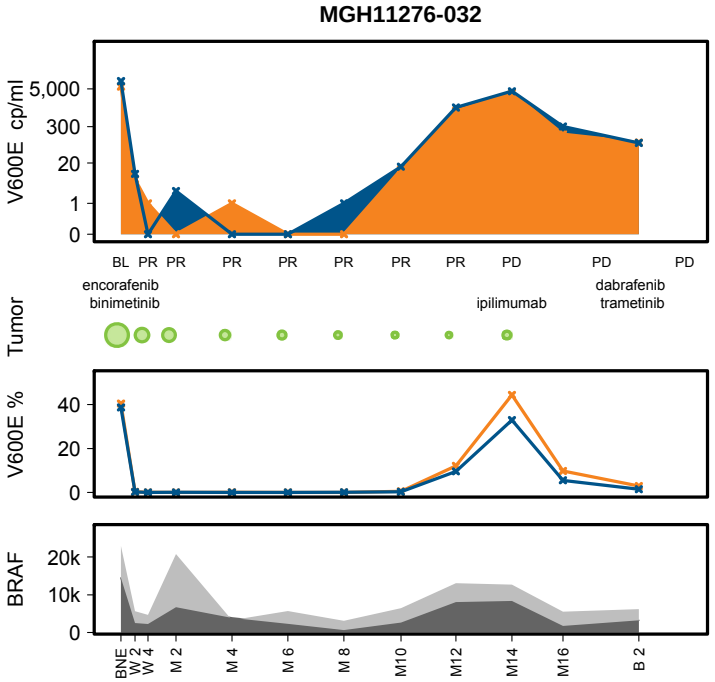






- Blood BRAF levels are reduced in all patients treated with BRAF inhibitor-based therapy
- Reduction in blood BRAF level is similar in patients treated with vemurafenib and dabrafenib + trametinib
- In at least a third to a half of patients, blood BRAF value increases in advance of radiographic evidence of PD

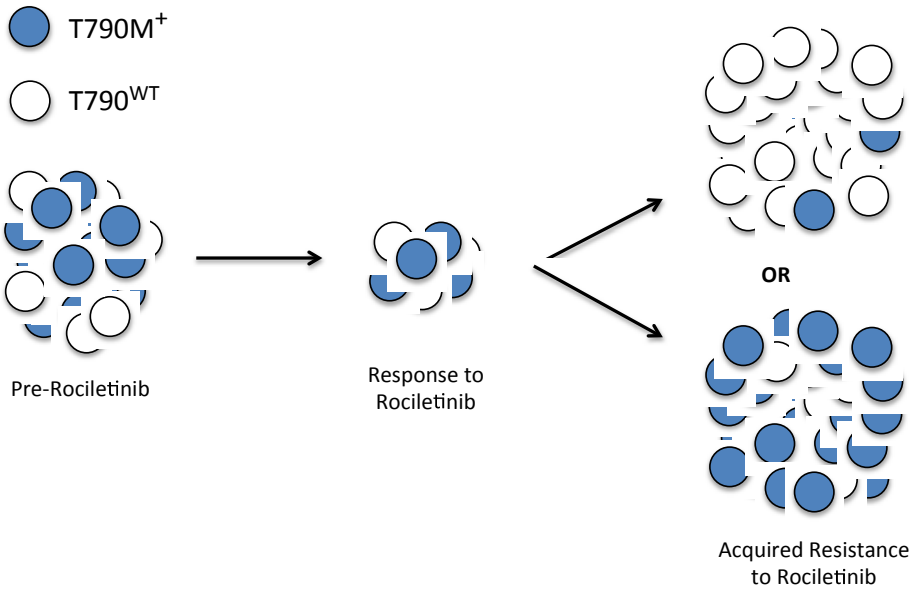
Panka et al. Mol Cancer Ther. 2014

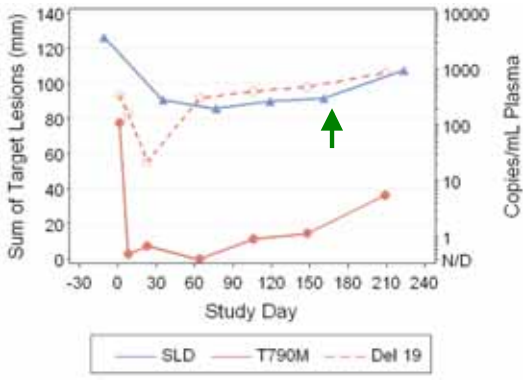


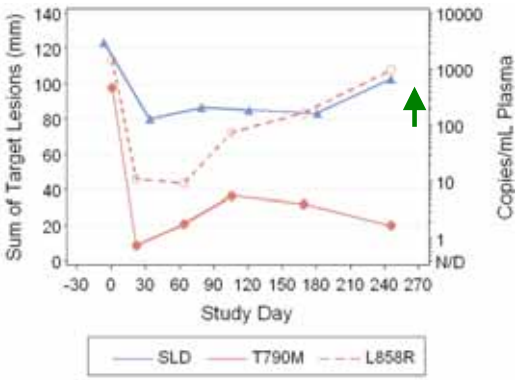
- BRAF blood levels can be measured in cfDNA and exoRNA in BRAF mutant, Stage IV melanoma (12/12 patients)
- Levels reduced in 11, and all 10 PRs
- Levels increases in 9 of 10 PRs at time of PD, and 5/10 ahead of imaging PD

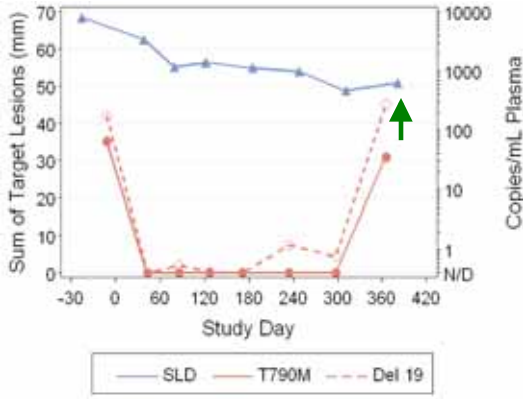
Sullivan et al. ASCO. 2015

Response and Resistance Monitoring Strategy









Longitudinal plasma ctDNA assessments demonstrate the emergence of T790M-positive and T790-wild type rociletinib resistance.

Piotrowska, et al. Cancer Discovery 2015

Concluding Thoughts

- Effective and transformative immunotherapy has been developed for the treatment of many malignancies
- An important and emerging issue is figuring out to whom we should be offering standard immunotherapy
- Blood-based biomarkers may help with patient selection
  - Serum protein quantification, exosomal RNA analysis, PDL1 expression (PBMCs)
- Rapid improvements in detection and quantification of oncogenic mutations in blood is changing how we diagnoses and treat patients
  - CTCs, cfDNA, exosomal RNA
- As genetic mechanisms of resistance to immunotherapies are described, the application of assays currently used in the targeted therapy setting to immunotherapy will be critical

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