

BCB 731:

Defense Against the Dark Arts



Optimist: Genetic basis for
clinical response to CTLA-4
blockade in melanoma

October 23rd, 2023



Background

Short history of cancer immunotherapy

CONTRIBUTION TO THE KNOWLEDGE OF SARCOMA.¹

By WILLIAM B. COLEY, M.D.,

OF NEW YORK.

I. A CASE OF PERIOSTEAL ROUND-CELLED SARCOMA OF THE METACARPAL BONE; AMPUTATION OF THE FOREARM; GEN-

1850s-1890s

Infection & fever =>
tumor regression?

1893

Coley's Toxins
(complete response in
~10% of sarcomas)

20th century

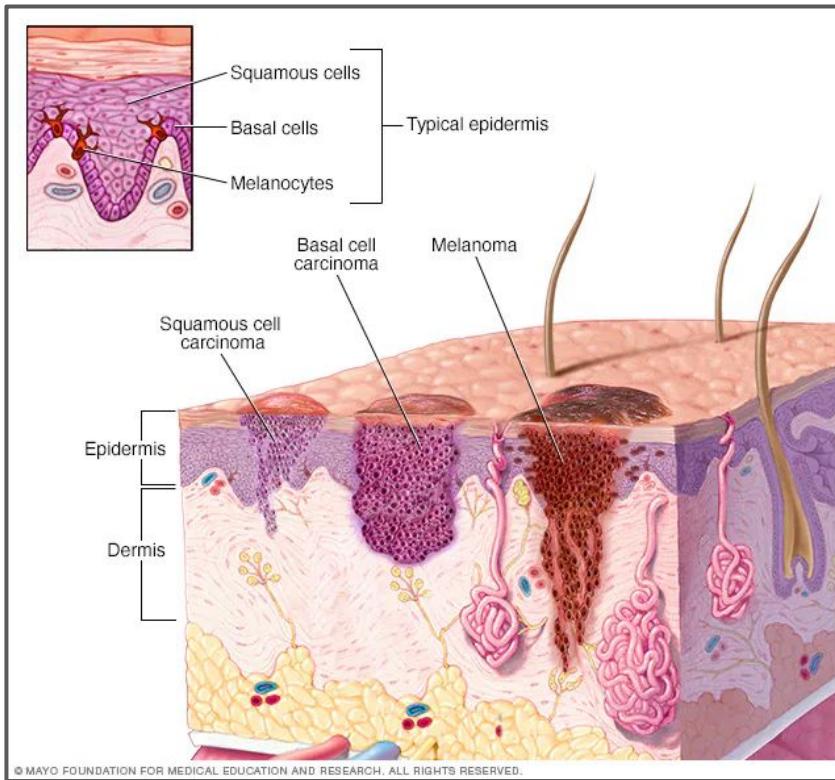
Dark Age of
radiation and
chemotherapy



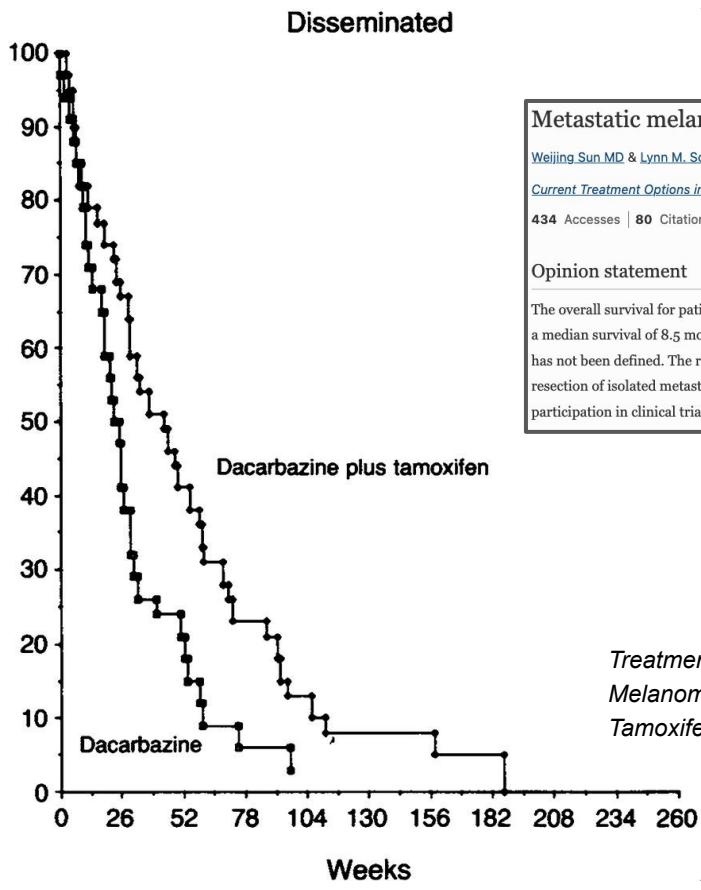
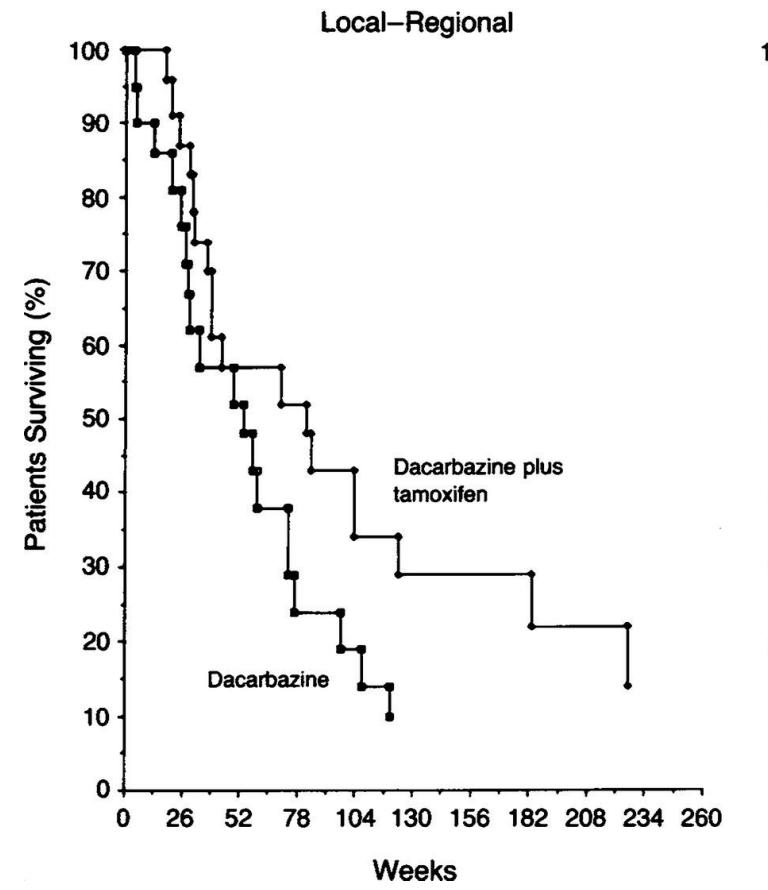
2010s

~20 approved cancer
immunotherapies

Melanoma



Metastatic Melanoma (Pre-2010s)



Metastatic melanoma

Weijing Sun MD & Lynn M. Schuchter MD

Current Treatment Options in Oncology 2, 193–202 (2001) | [Cite this article](#)

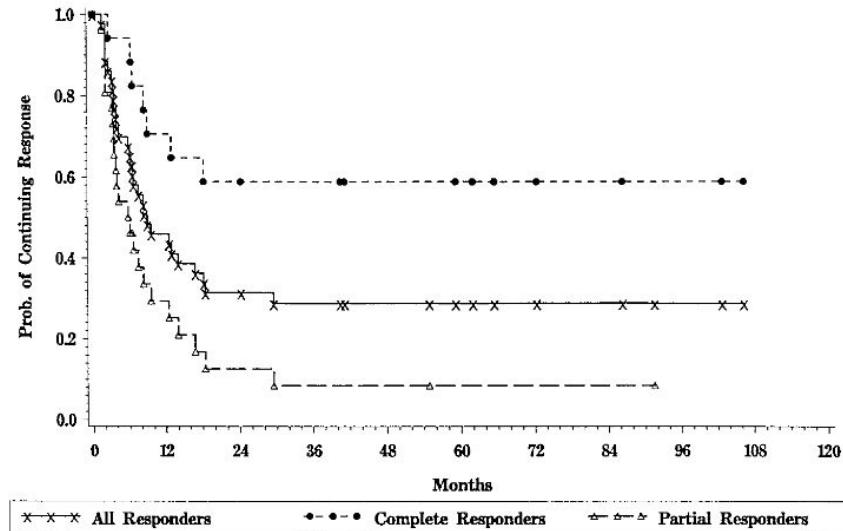
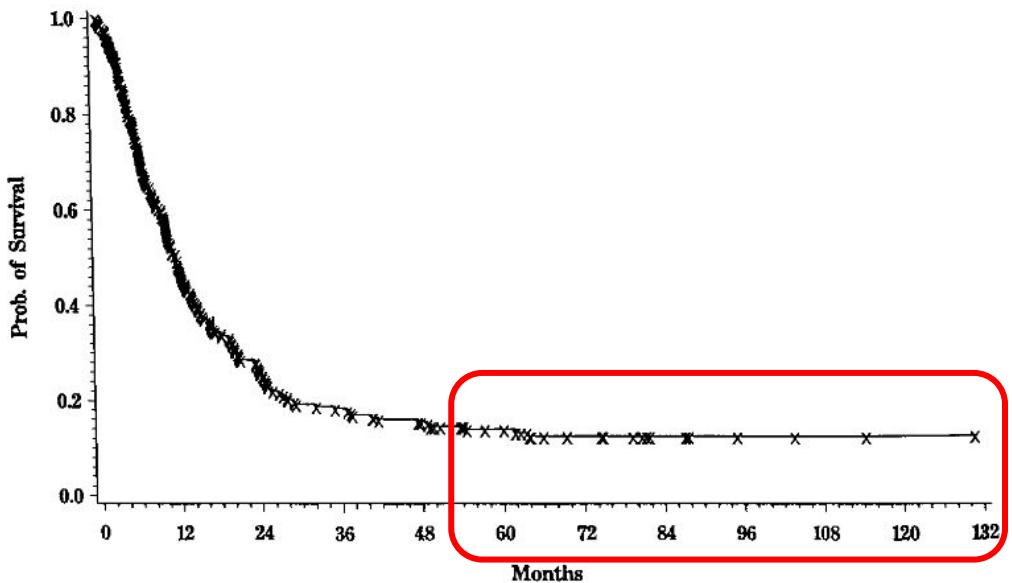
434 Accesses | 80 Citations | [Metrics](#)

Opinion statement

The overall survival for patients with metastatic melanoma ranges from 4.7 to 11 months, with a median survival of 8.5 months. Standard treatment for patients with metastatic melanoma has not been defined. The range of treatment options includes close observation, surgical resection of isolated metastases, therapy with dacarbazine, combination chemotherapy, and participation in clinical trials.

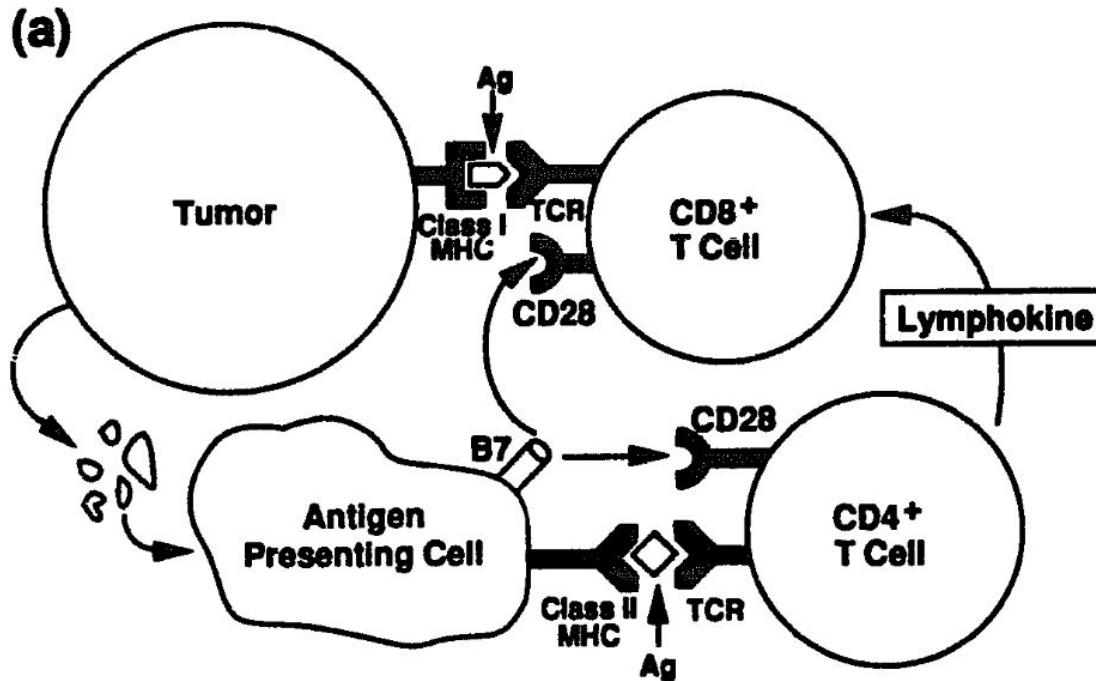
Treatment of Metastatic Malignant Melanoma with Dacarbazine plus Tamoxifen (1992)

Glimpse of Hope in (IL-2) Immunotherapy



High-Dose Recombinant Interleukin 2 Therapy for Patients With Metastatic Melanoma: Analysis of 270 Patients Treated Between 1985 and 1993

What mediates anti-tumor immunity?



Costimulation of T cells for tumor immunity (1993)

CTLA-4 (and the other T-cell checkpoints)

NATURE VOL. 328 16 JULY 1987

LETTERS TO NATURE

267

A new member of the immunoglobulin superfamily—CTLA-4

Jean-François Brunet, François Denizot,
Marie-Françoise Luciani, Magali Roux-Dosseto^{*‡},
Marie Suzan, Marie-Geneviève Mattei[†]
& Pierre Golstein

Centre d'Immunologie INSERM-CNRS de Marseille-Luminy,
Case 906, 13288 Marseille Cedex 9, France

^{*}INSERM U.119, 27, Boulevard Léon Roux, 13009 Marseille, France
[†]INSERM U.242, CHU Timone, 13385 Marseille Cedex 5, France

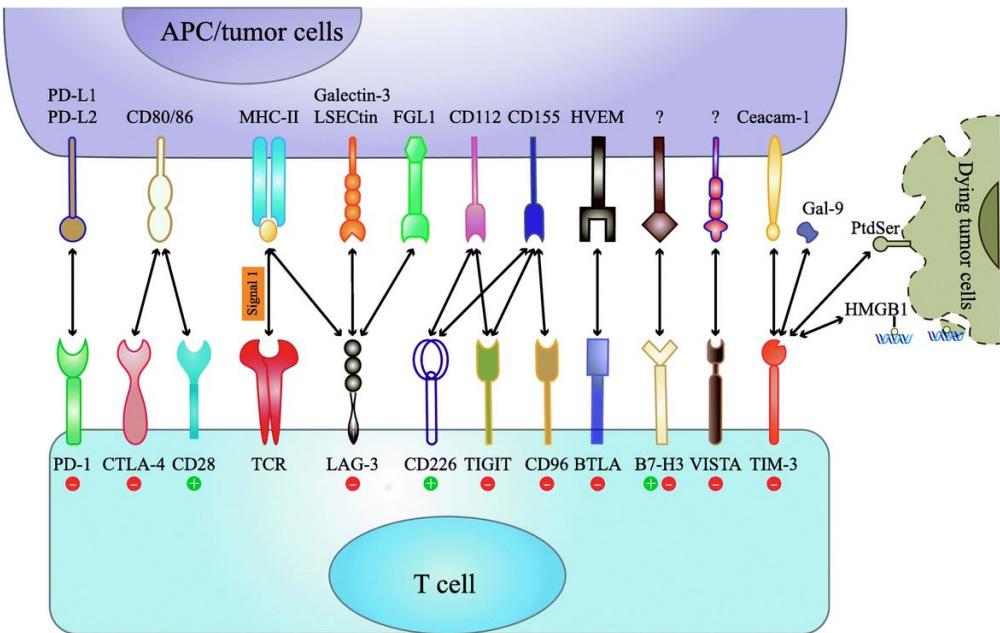
[‡] Present address: UA CNRS 1175, Faculté de Médecine Secteur Nord,
13326 Marseille Cedex 15, France

The immunoglobulin superfamily is a group of proteins, each made of one or several domains sharing key structural features with either the variable (V) or the constant (C) immunoglobulin domains^{1,2}. It includes such functionally important members as the immunoglobulins themselves, major histocompatibility complex (MHC) class I and class II and T-cell receptor (TCR) molecules. Several members of this superfamily are expressed on lymphocytes where they are membrane-bound and capable of interactions with other members of the family, thus taking part in cell-cell recognition. In screening mouse cytolytic-T-cell-derived cDNA libraries, we came across cDNA clones defining a sequence, CTLA-4, which could encode a 223-amino-acid protein clearly belonging to the immunoglobulin superfamily. It consists of one V-like domain flanked by two hydrophobic regions, one of which has a structure suggestive of membrane anchoring. CTLA-4 is mainly expressed in activated lymphocytes and is coinduced with T-cell-mediated cytotoxicity in inducible models of this process. The mouse *ctla-4* gene maps to band C of chromosome 1.

We previously reported the construction of a subtracted cytolytic-T-cell(Tc)-derived complementary DNA library of about 8,300 clones, from which three distinct cytolytic T-cell-associated sequences, CTLA-1, CTLA-2 and CTLA-3, were iso-

lated by conventional differential screenings³. CTLA-1 and CTLA-3 encode distinct serine-esterases, also detected, using similar approaches, as CCP-1 (ref. 4) and H factor⁵, respectively; CTLA-2 encodes a previously undescribed protein product (F.D. *et al.*, in preparation). Further screening of the same subtracted library with a subtracted (Tc-B) cDNA probe allowed the detection of 113 additional clones which had not detectably hybridized with a total Tc-derived cDNA probe. These clones were subjected to successive rounds of screening with several other Tc cDNA probes subtracted against messenger RNA derived from either the B lymphoma M12.4.1, or the EL4 thymoma, or thymocytes. The positive, thus still apparently Tc-specific, clones were then depleted of copies of CTLA-1, -2 and -3 by probing with a pool of inserts representative of these families. The inserts of the 17 cDNA clones left at that stage were used as probes on Northern blots of a panel of RNAs. Only one clone, M17G7, survived this ultimate screening for Tc specificity in that it detected a 2-kilobase (kb) transcript in RNA from the original KB5C20 Tc clones and not from other, non-Tc cells. The M17G7 cDNA insert defines CTLA-4.

A study of the tissue distribution of the CTLA-4-2 kb transcript (Fig. 1) revealed its presence in each of the Tc or Tc-containing populations tested (two clones: KB5C20 and A15.1.17, albeit at a low level for the latter; and three *in vitro* or *in vivo* primary populations: mixed leucocyte culture cells, concanavalin-A-activated blasts and allo-sensitized peritoneal exudate lymphocytes). In addition, like the CTLA-1, -2 and -3 transcripts³, the CTLA-4 transcript was coinduced with cytotoxicity in two systems: on incubation of the constitutively growing cytolytic hybridoma PC60 (ref. 6) with interleukin-containing supernatants and on incubation of concanavalin-A-pulsed thymocytes with an interleukin-2-containing supernatant. A weaker signal was detected in lipopolysaccharide(LPS)-activated blasts (the significance of which is unclear due to the likely heterogeneity of this population) and non-induced PC60 cells, and a very weak one in non-activated thymocytes (Fig. 1) and in non-experimentally-activated peripheral lymphocytes, that is column-purified or unpurified normal spleen cells (data not shown). EL4, the T helper hybridoma T14-117, NK-cell-



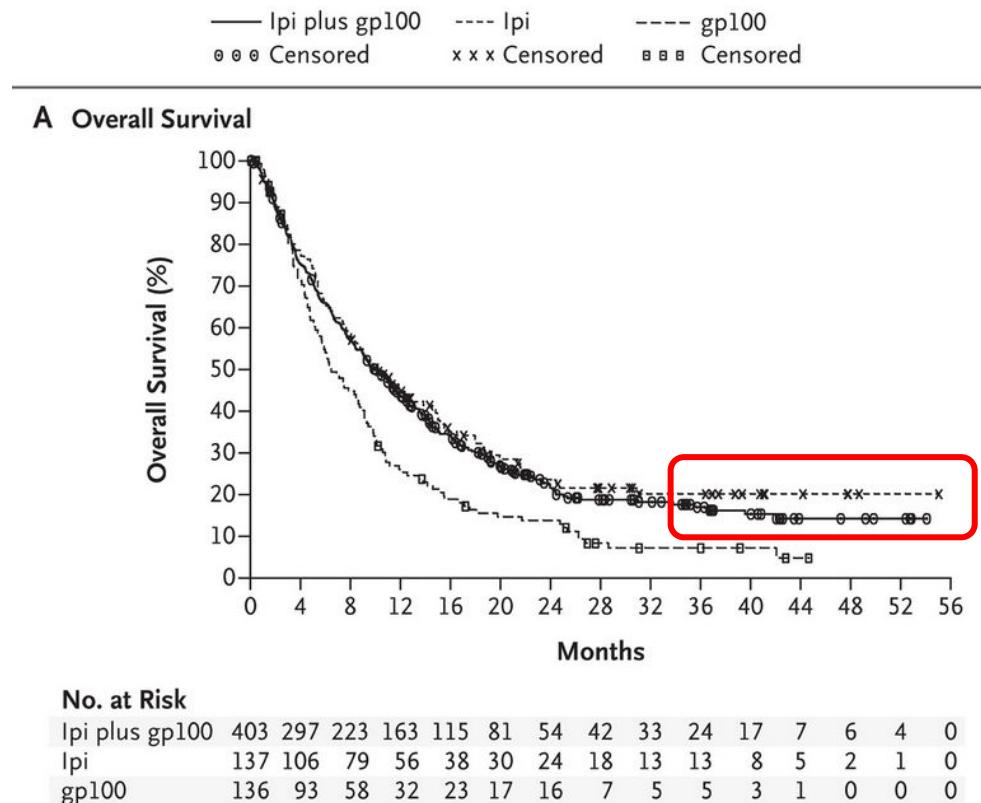
Novel immune checkpoint targets: moving beyond PD-1 and CTLA-4

CTLA-4 Blockade for Metastatic Melanoma

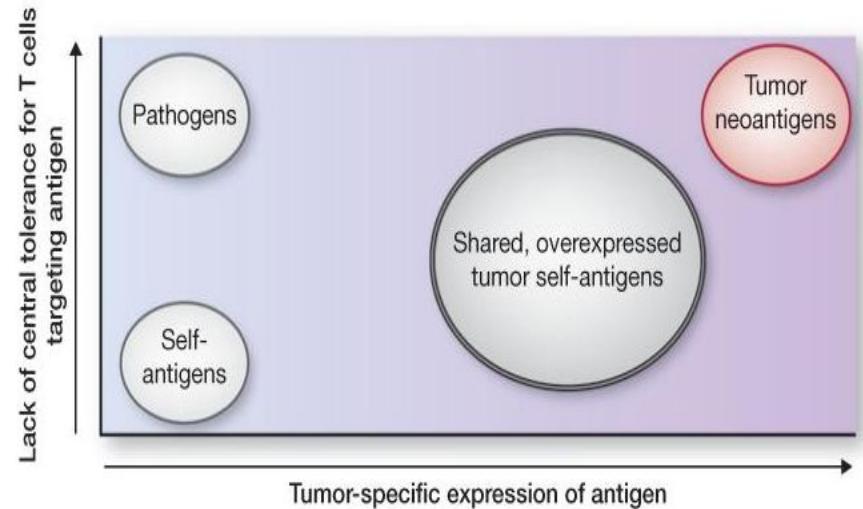
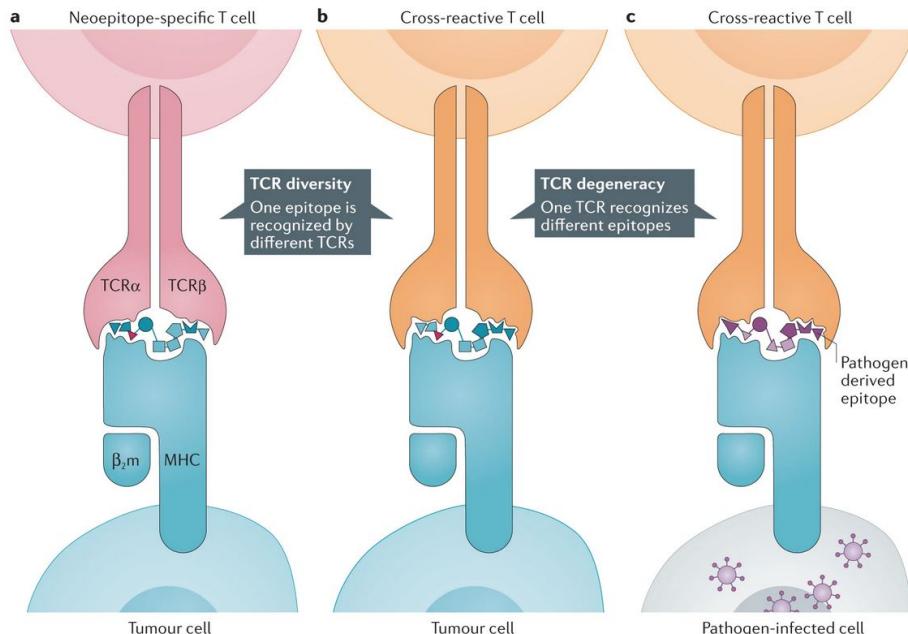
PATIENTS

Patients were eligible for inclusion in the study if they had a diagnosis of unresectable stage III or IV melanoma and had received a previous therapeutic regimen containing one or more of the following: dacarbazine, temozolomide, fotemustine, carboplatin, or interleukin-2. Other inclusion criteria were

Improved Survival with Ipilimumab in Patients with Metastatic Melanoma



What do cytotoxic (CD8+) T-cells recognize on cancer cells?



Identification of neoantigens for individualized therapeutic cancer vaccines

Getting Personal with Neoantigen-Based Therapeutic Cancer Vaccines

1

Obtain tumor material



2

Identify tumor-specific mutations within expressed genes

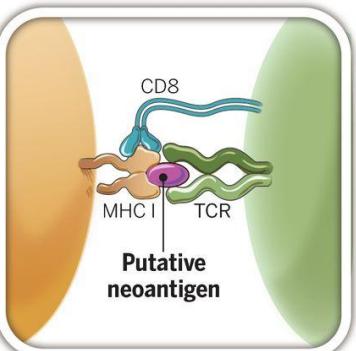
3

Filter *in silico*

Filter by MS analysis

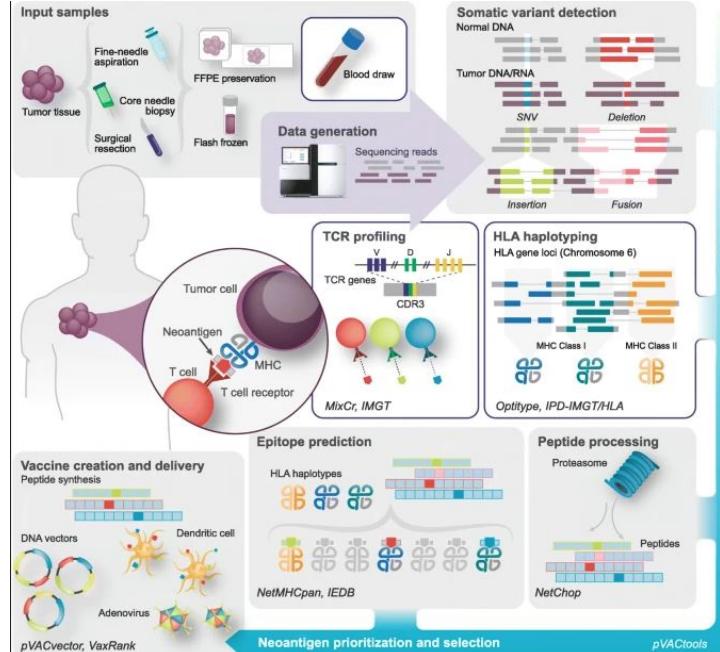
4

Assess T cell recognition



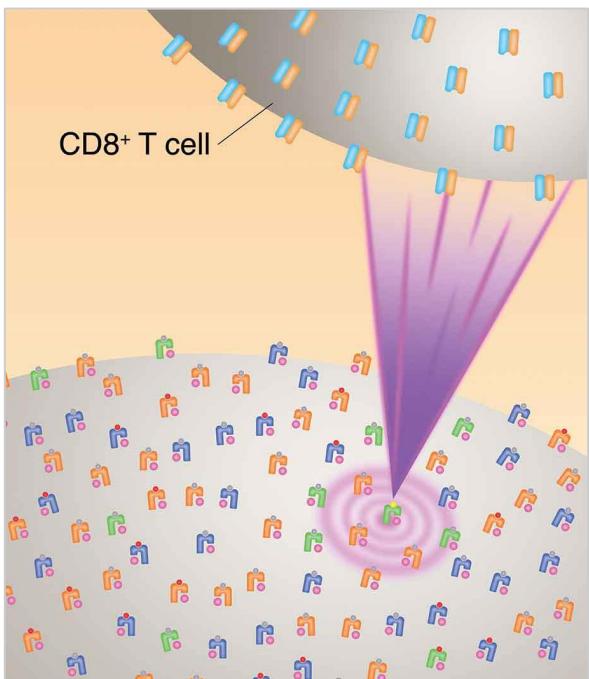
Neoantigens in cancer immunotherapy

How to identify neoantigens?

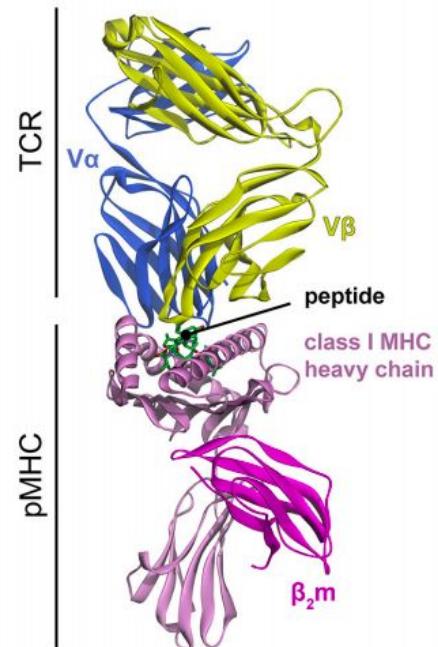


Best practices for bioinformatic characterization of neoantigens for clinical utility

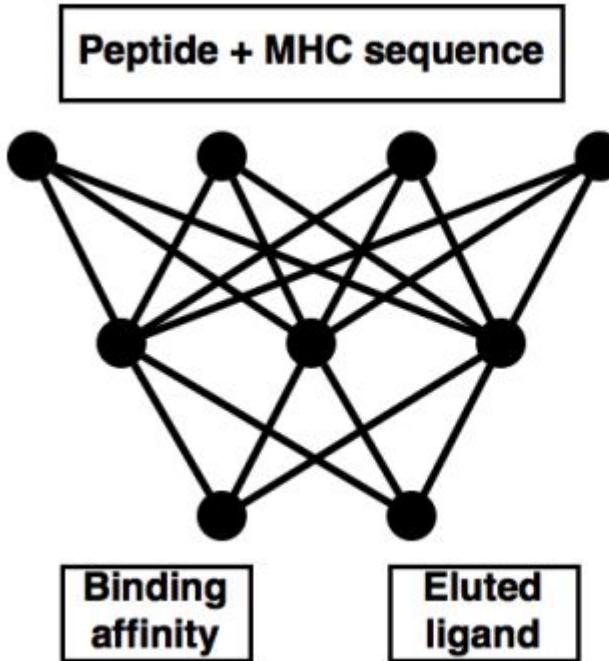
MHC binding prediction



Lost in the crowd: identifying targetable MHC class I neoepitopes for cancer immunotherapy



Using Global Analysis to Extend the Accuracy and Precision of Binding Measurements with T cell Receptors and Their Peptide/MHC Ligands



NetMHCpan 4.0

The Paper

ORIGINAL ARTICLE

Genetic Basis for Clinical Response to CTLA-4 Blockade in Melanoma

Alexandra Snyder, M.D., Vladimir Makarov, M.D., Taha Merghoub, Ph.D.,
Jianda Yuan, M.D., Ph.D., Jesse M. Zaretsky, B.S., Alexis Desrichard, Ph.D.,
Logan A. Walsh, Ph.D., Michael A. Postow, M.D., Phillip Wong, Ph.D.,
Teresa S. Ho, B.S., Travis J. Hollmann, M.D., Ph.D., Cameron Bruggeman, M.A.,
Kasthuri Kannan, Ph.D., Yanyun Li, M.D., Ph.D., Ceyhan Elipenahli, B.S.,
Cailian Liu, M.D., Christopher T. Harbison, Ph.D., Lisu Wang, M.D.,
Antoni Ribas, M.D., Ph.D., Jedd D. Wolchok, M.D., Ph.D.,
and Timothy A. Chan, M.D., Ph.D.

Meet the Cohorts

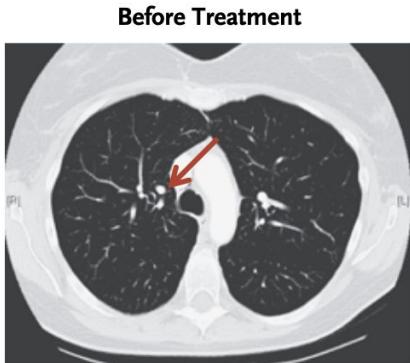
- Combined trials of two different anti-CTLA-4 antibodies
 - ipilimumab
 - tremelimumab
- 64 patients with WES
 - 25 “discovery”
 - 39 “validation”
- Identified mutations + predicted neoantigens

Characteristic	Discovery Set		Validation Set	
	Long-Term Benefit (N=11)	Minimal or No Benefit (N=14)	Long-Term Benefit (N=25)	Minimal or No Benefit (N=14)
	Table 1. Clinical Characteristics of the Patients in the Discovery and Validation Sets, According to Clinical Benefit from Therapy.			
Age at start of treatment — yr				
Median	63	60	66	57
Range	39–70	48–79	33–90	18–74
Sex — no. of patients (%)				
Female	3 (27)	8 (57)	9 (36)	5 (36)
Male	8 (73)	6 (43)	16 (64)	9 (64)
Disease origin — no. of patients (%)				
Acral	0	3 (21)	1 (4)	1 (7)
Uveal	0	0	1 (4)	0
Cutaneous	10 (91)	8 (57)	15 (60)	11 (79)
Unknown primary	1 (9)	3 (21)	3 (12)	0
Not available	0	0	5 (20)	2 (14)
BRAF or NRAS mutation — no. of patients (%)				
No	1 (9)	6 (43)	17 (68)	11 (79)
Yes	10 (91)	8 (57)	8 (32)	3 (21)
Lactate dehydrogenase level at start of therapy — no. of patients (%)				
Normal	8 (73)	8 (57)	8 (32)	9 (64)
Above normal	2 (18)	5 (36)	3 (12)	3 (21)
Not available	1 (9)	1 (7)	14 (56)	2 (14)
Duration of response to therapy — wk				
Median	59	14	130	11
Range	42–361	11–23	64–376	3–29
Previous therapies — no.*				
Median	1	1	0	0
Range	0–3	0–2	0–2	0–3
Melanoma stage at time of diagnosis — no. of patients (%)				
IIC	0	0	3 (12)	0
M1a	0	1 (7)	4 (16)	1 (7)
M1b	5 (45)	1 (7)	2 (8)	3 (21)
M1c	6 (55)	12 (86)	16 (64)	10 (71)
Overall survival — yr†				
Median	4.4	0.9	3.3	0.8
Range	2.0–6.9	0.4–2.7	1.6–7.2	0.2–2.1

Defining α CTLA-4 Benefit

Benefit: stable or decreased tumor size for 6+ months after start of treatment

B Patient with Progressive Disease



After Treatment

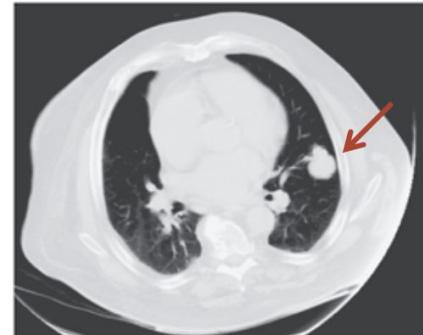
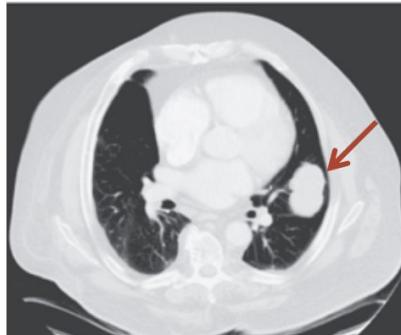
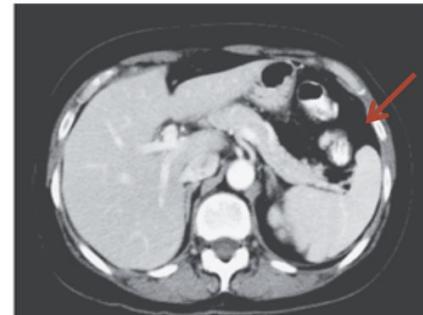


A Patients with a Long-Term Clinical Benefit from Therapy

Before Treatment

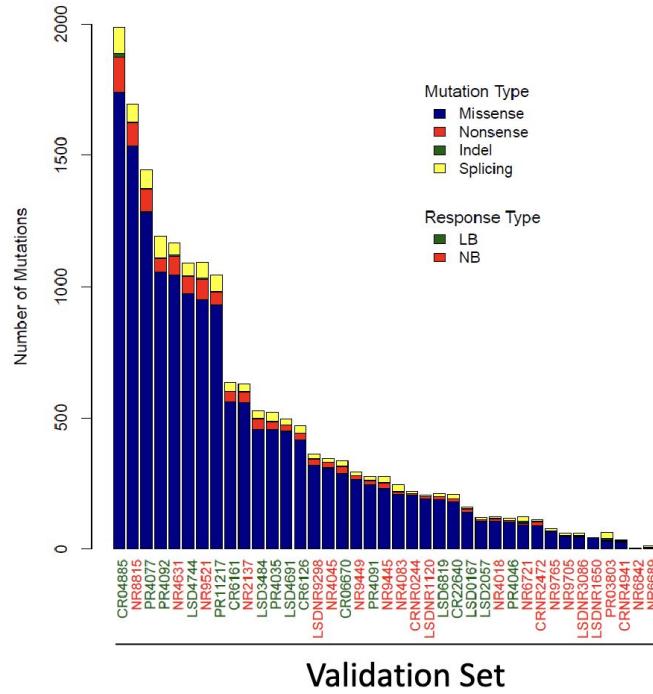
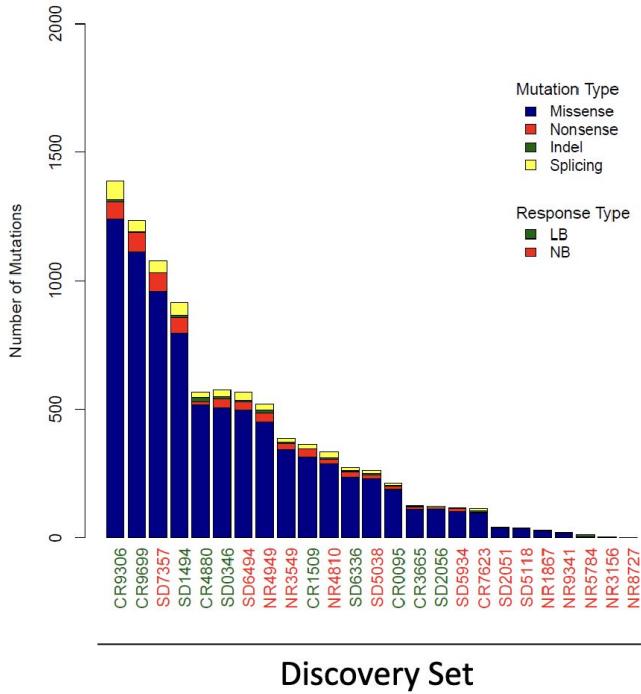


After Treatment



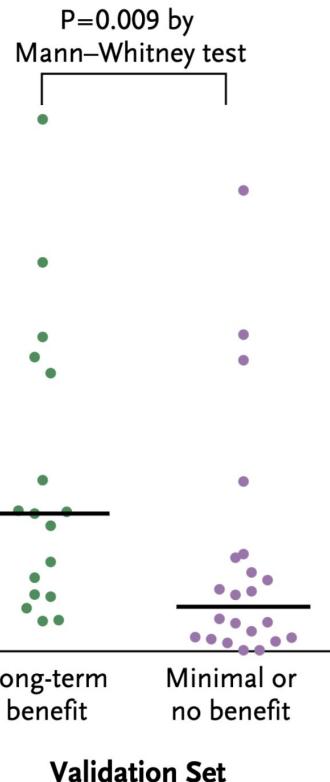
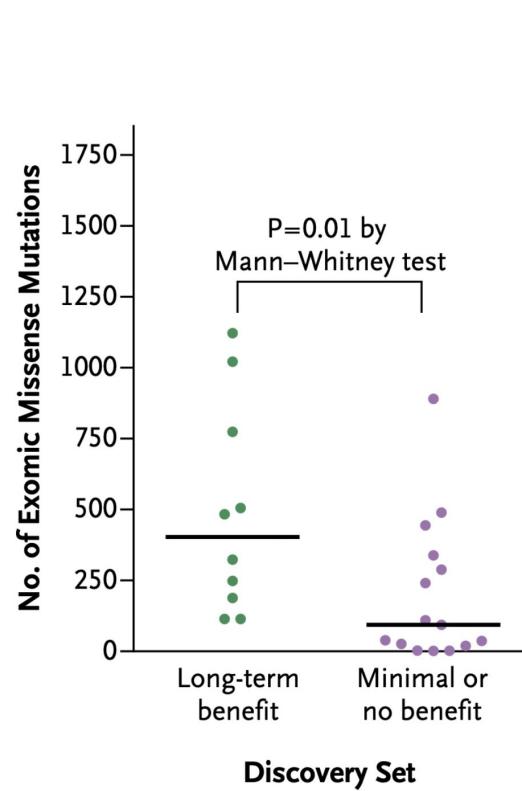
WES Mutations

20

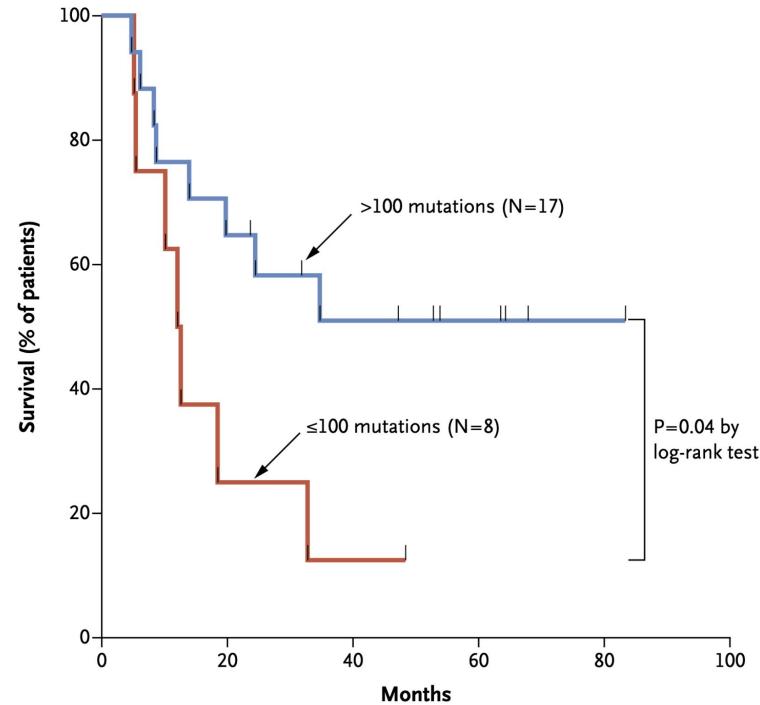


Mutation Counts Predict Benefit

A Mutational Load



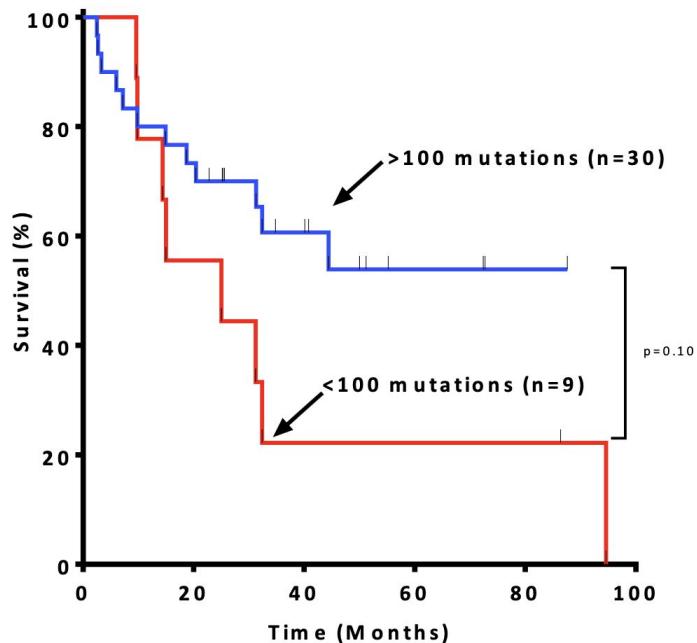
B Survival in Discovery Set



...in the Validation Set too

Figure S3A

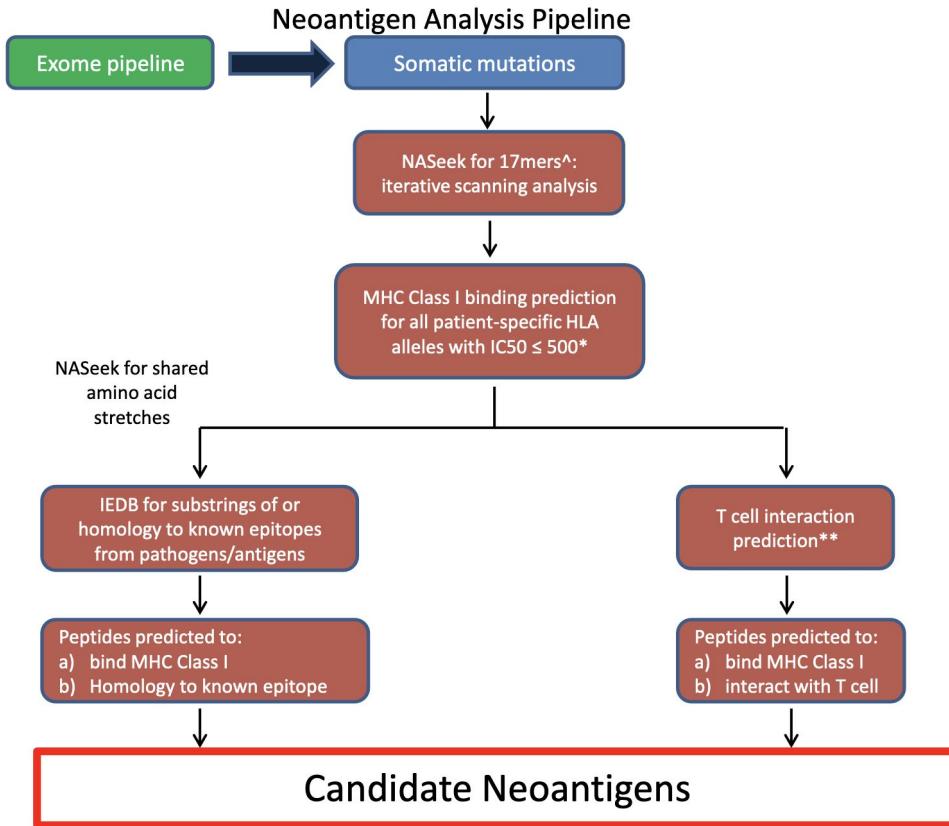
23



Neoantigen Signature Pipeline

Figure S4

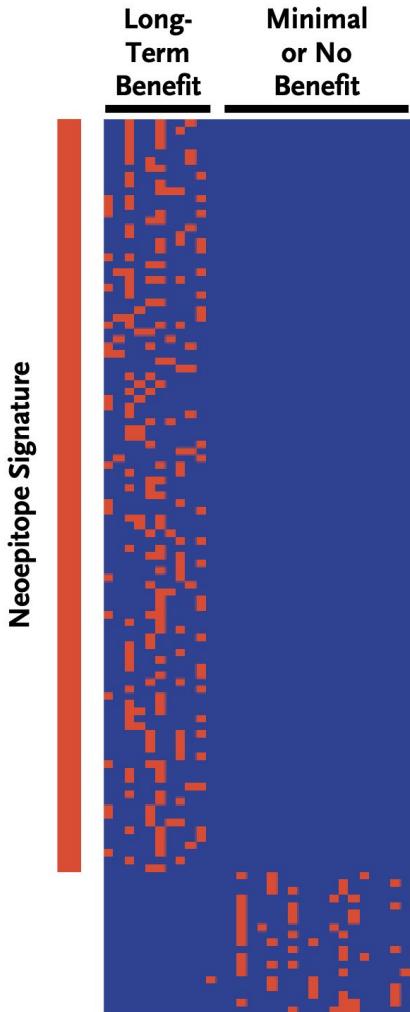
25



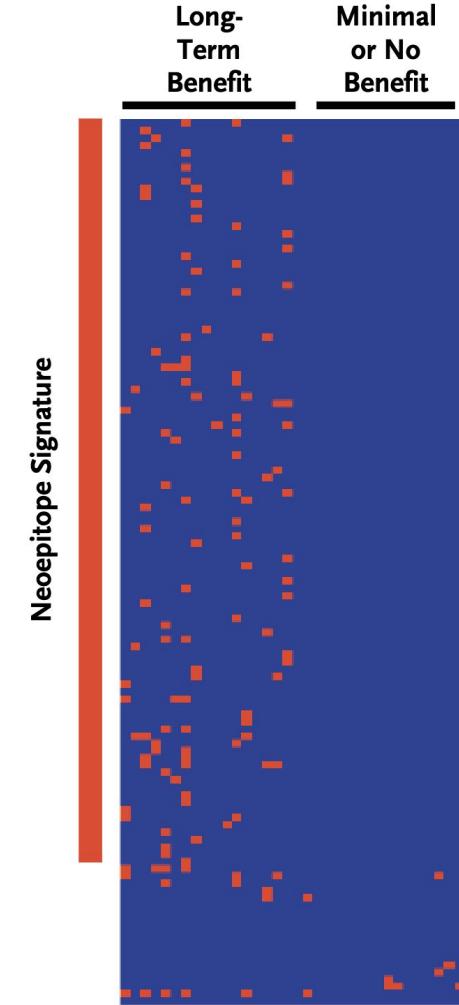
Neoantigen Signature

4mer	Sample	Chr	Position	Gene	Mutation	Amino Acid	WTSeq	MTSeq
AALL								
AALL	CR9699	chrX	14708901	GLRA2	c.G1000A	p.E334K	fvfaallEy	fvfaallKy
AALL	SD1494	chr9	94486131	ROR2	c.C2645T	p.S882F	Smadraall	Fmadraall
AALL	CR0095	chrX	11197491	ARHGAP6	c.G1411A	p.E471K	aallkEfIrl	aallkKfIrl
AALL	CR04885	chr19	8389389	KANK3	c.G2326A	p.D776N	Devaallha	Nevaallha
AALL	PR11217	chr19	50209622	CPT1C	c.C1295T	p.S432L	dpaallday	dpaallday
AATA								
AATA	CR9699	chr22	32555082	C22orf42	c.C121T	p.P41S	etvaataPa	etvaataSa
AATA	SD1494	chr20	61528063	DIDO1	c.C1874T	p.S625F	apaataaaS	apaaaataaaF
AATA	CR4880	chrX	24382384	FAM48B1	c.G1507A	p.A503T	aiaaaAaaa	aiaaaTaaa
AATA	LSD3484	chr20	44580928	ZNF335	c.C3047T	p.S1016L	saataaSkk	saataaLkk
AFPS								
AFPS	CR9699	chr10	63852764	ARID5B	c.C3542T	p.S1181F	afpsqlsS	afpsqlsF
AFPS	SD1494	chr7	12409653	VWDE	c.C2279T	p.P760L	fpPlfafps	fpLfafps
AFPS	LSD4691	chr3	188202427	LPP	c.241C>T	p.L81F	aLpsisgnf	aFpsisgnf
AFPS	PR4092	chr2	237076596	GBX2	c.C19T	p.P7S	aafPslmm	aafPSSLmm
AGAA								
AGAA	CR9699	chr14	94909083	SERPINA11	c.C1129T	p.L377F	teagaasgL	teagaasgF
AGAA	SD1494	chr19	10946865	TMED1	c.G3A	p.M1I	Mmaagaala	Imaagaala
AGAA	LSD3484	chr7	27282896	EVX1	c.G247A	p.E83K	Epqvagaam	Kpqvagaam

A Neoepitopes in Discovery Set



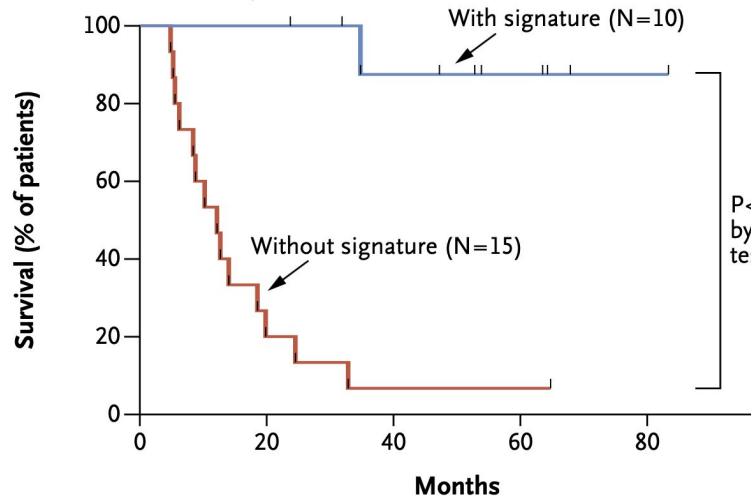
B Neoepitopes in Validation Set



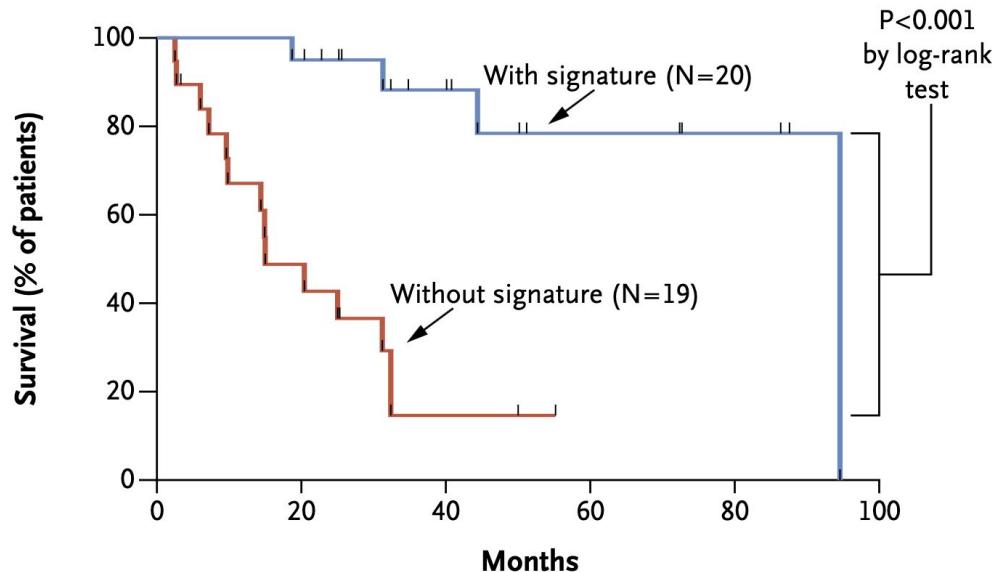
Neoantigen Signature Predictive of Benefit

Neoantigen Signature KM Curves

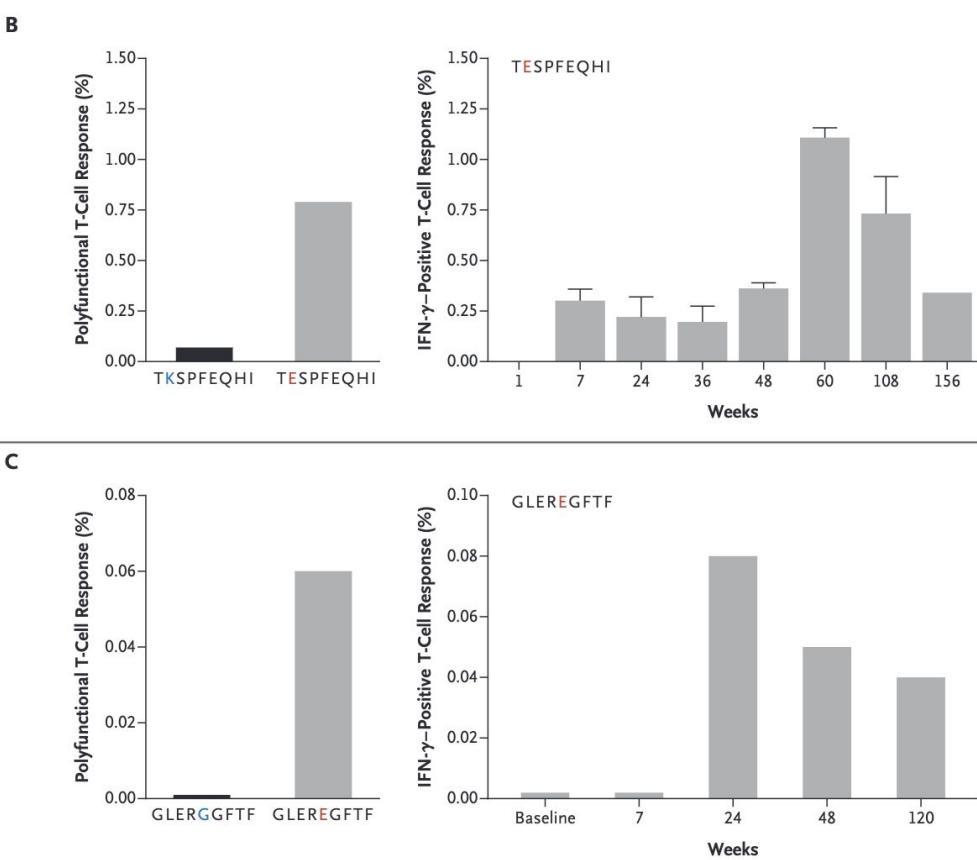
C Survival in Discovery Set



D Survival in Validation Set



Validation



 *Fin*

Prologue: what causes depression?

NHS

Search

My account

Health A-Z Live Well Mental health Care and support Pregnancy NHS services

Home > Medicines A to Z > Fluoxetine (Prozac)

About fluoxetine

Fluoxetine is a type of antidepressant known as a selective serotonin reuptake inhibitor (SSRI). It's often used to treat [depression](#), and sometimes [obsessive compulsive disorder](#) and [bulimia](#).

It works by increasing the levels of serotonin in the brain. [Serotonin is thought to have a good influence on mood, emotion and sleep.](#)

<https://www.nhs.uk/medicines/fluoxetine-prozac/about-fluoxetine/>

HOW PROZAC CAN HELP

How it Works

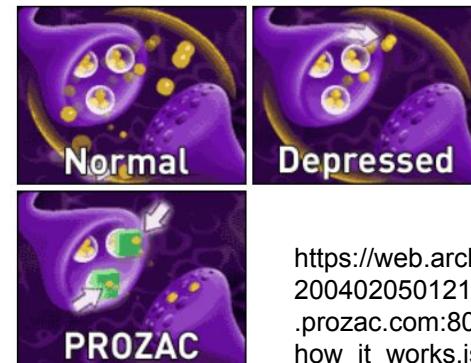
Depression is not fully understood, but a growing amount of evidence supports the view that people with depression have an imbalance of the brain's neurotransmitters, the chemicals that allow nerve cells in the brain to communicate with each other. Many scientists believe that an imbalance in serotonin, one of these neurotransmitters, may be an important factor in the development and severity of depression.

PROZAC may help to correct this imbalance by increasing the brain's own supply of serotonin.

Some other antidepressant medicines appear to affect several neurotransmitters in addition to serotonin. PROZAC selectively affects only serotonin.

While PROZAC cannot be said to "cure" depression, it does help to control the symptoms of depression, allowing many people with depression to feel better and return to normal functioning. [See graphics below]

Below you will find links to animations of the serotonin system within the brain.



https://web.archive.org/web/20040205012110/http://www.prozac.com:80/how_prozac/how_it_works.jsp

...and yet...

SYSTEMATIC REVIEW OPEN

The serotonin theory of depression: a systematic umbrella review of the evidence

Joanna Moncrieff^{1,2}✉, Ruth E. Cooper³, Tom Stockmann⁴, Simone Amendola⁵, Michael P. Hengartner⁶ and Mark A. Horowitz^{1,2}

© The Author(s) 2022

Design Reanalysis of a systematic review, with meta-analyses.

Data sources 522 trials (116 477 participants) as reported in the systematic review by Cipriani *et al* and clinical study reports for 19 of these trials.

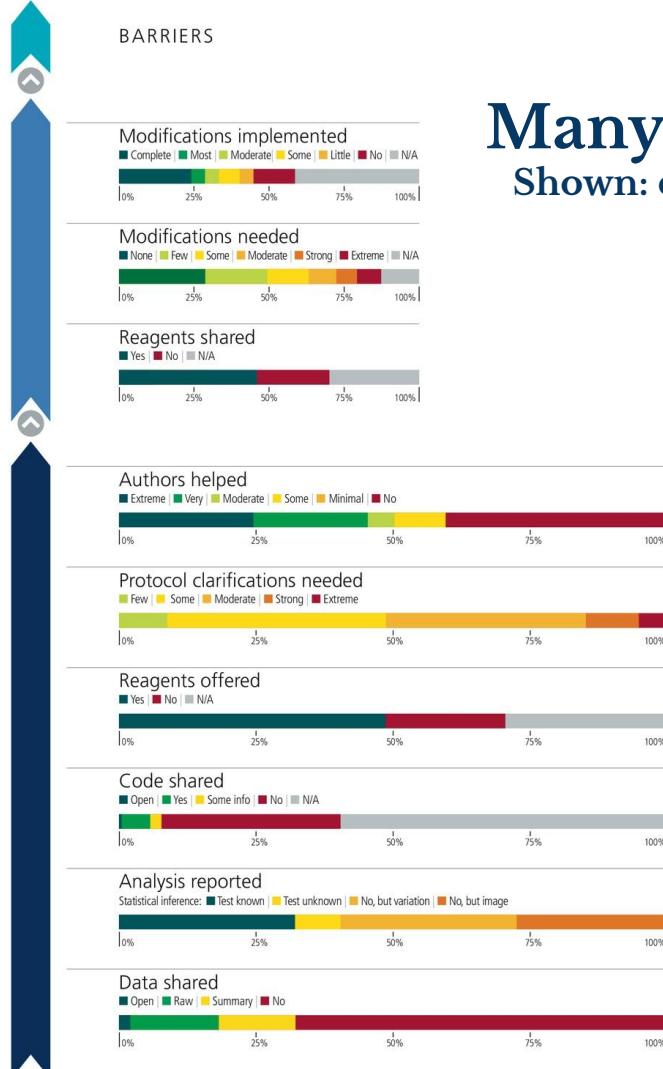
Analysis We used the Cochrane Handbook's risk of bias tool and the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach to evaluate the risk of bias and the certainty of evidence, respectively. The impact of several study characteristics and publication status was estimated using pairwise subgroup meta-analyses.

Results Several methodological limitations in the evidence base of antidepressants were either unrecognised or underestimated in the systematic review by Cipriani *et al*. The effect size for antidepressants versus placebo on investigator-rated depression symptom scales was higher in trials with a 'placebo run-in' study design compared with trials without a placebo run-in design ($p=0.05$). The effect size of antidepressants was higher in published trials compared with unpublished trials ($p<0.0001$). The outcome data reported by Cipriani *et al* differed from the clinical study reports in 12 (63%) of 19 trials. The certainty of the evidence for the placebo-controlled comparisons should be very low according to GRADE due to a high risk of bias, indirectness of the evidence and publication bias. The mean difference between antidepressants and placebo on the 17-item Hamilton depression rating scale (range 0–52 points) was 1.97 points (95% CI 1.74 to 2.21).

Conclusions The evidence does not support definitive conclusions regarding the benefits of antidepressants for depression in adults. It is unclear whether antidepressants are more efficacious than placebo.

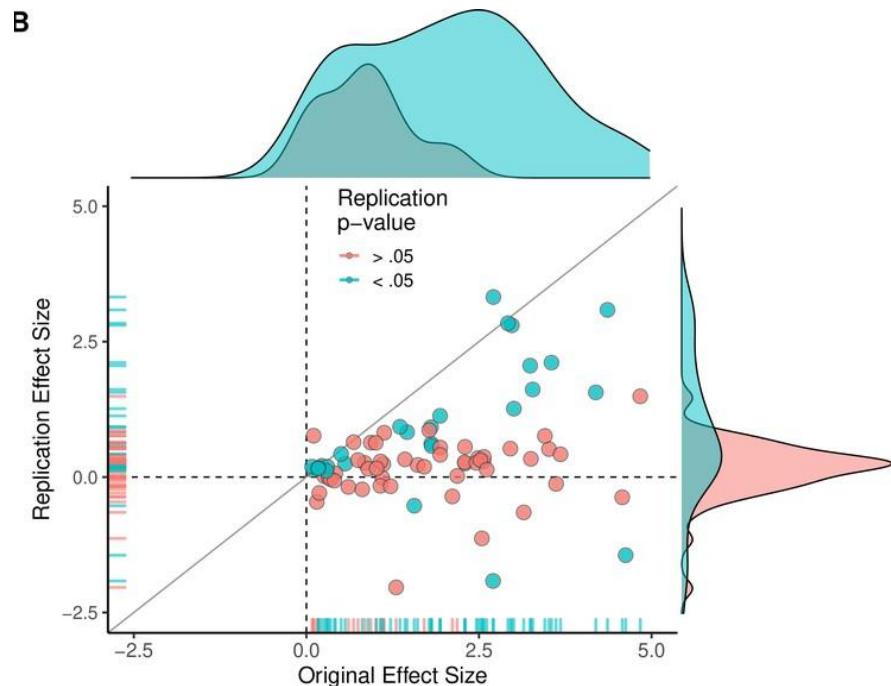
The serotonin hypothesis of depression is still influential. We aimed to synthesise and evaluate evidence on whether depression is associated with lowered serotonin concentration or activity in a systematic umbrella review of the principal relevant areas of research. PubMed, EMBASE and PsycINFO were searched using terms appropriate to each area of research, from their inception until December 2020. Systematic reviews, meta-analyses and large data-set analyses in the following areas were identified: serotonin and serotonin metabolite, 5-HIAA, concentrations in body fluids; serotonin 5-HT_{1A} receptor binding; serotonin transporter (SERT) levels measured by imaging or at post-mortem; tryptophan depletion studies; SERT gene associations and SERT gene-environment interactions. Studies of depression associated with physical conditions and specific subtypes of depression (e.g. bipolar depression) were excluded. Two independent reviewers extracted the data and assessed the quality of included studies using the AMSTAR-2, an adapted AMSTAR-2, or the STREGA for a large genetic study. The certainty of study results was assessed using a modified version of the GRADE. We did not synthesise results of individual meta-analyses because they included overlapping studies. The review was registered with PROSPERO (CRD42020207203). 17 studies were included: 12 systematic reviews and meta-analyses, 1 collaborative meta-analysis, 1 meta-analysis of large cohort studies, 1 systematic review and narrative synthesis, 1 genetic association study and 1 umbrella review. Quality of reviews was variable with some genetic studies of high quality. Two meta-analyses of overlapping studies examining the serotonin metabolite, 5-HIAA, showed no association with depression (largest $n = 1002$). One meta-analysis of cohort studies of plasma serotonin showed no relationship with depression, and evidence that lowered serotonin concentration was associated with antidepressant use ($n = 1869$). Two meta-analyses of overlapping studies examining the 5-HT_{1A} receptor (largest $n = 561$), and three meta-analyses of overlapping studies examining SERT binding (largest $n = 1845$) showed weak and inconsistent evidence of reduced binding in some areas, which would be consistent with increased synaptic availability of serotonin in people with depression, if this was the original, causal abnormality. However, effects of prior antidepressant use were not reliably excluded. One meta-analysis of tryptophan depletion studies found no effect in most healthy volunteers ($n = 566$), but weak evidence of an effect in those with a family history of depression ($n = 75$). Another systematic review ($n = 342$) and a sample of ten subsequent studies ($n = 407$) found no effect in volunteers. No systematic review of tryptophan depletion studies has been performed since 2007. The two largest and highest quality studies of the SERT gene, one genetic association study ($n = 115,257$) and one collaborative meta-analysis ($n = 43,165$), revealed no evidence of an association with depression, or of an interaction between genotype, stress and depression. The main areas of serotonin research provide no consistent evidence of there being an association between serotonin and depression, and no support for the hypothesis that depression is caused by lowered serotonin activity or concentrations. Some evidence was consistent with the possibility that long-term antidepressant use reduces serotonin concentration.

COMPLETED
50 experiments



Many published results are not useful

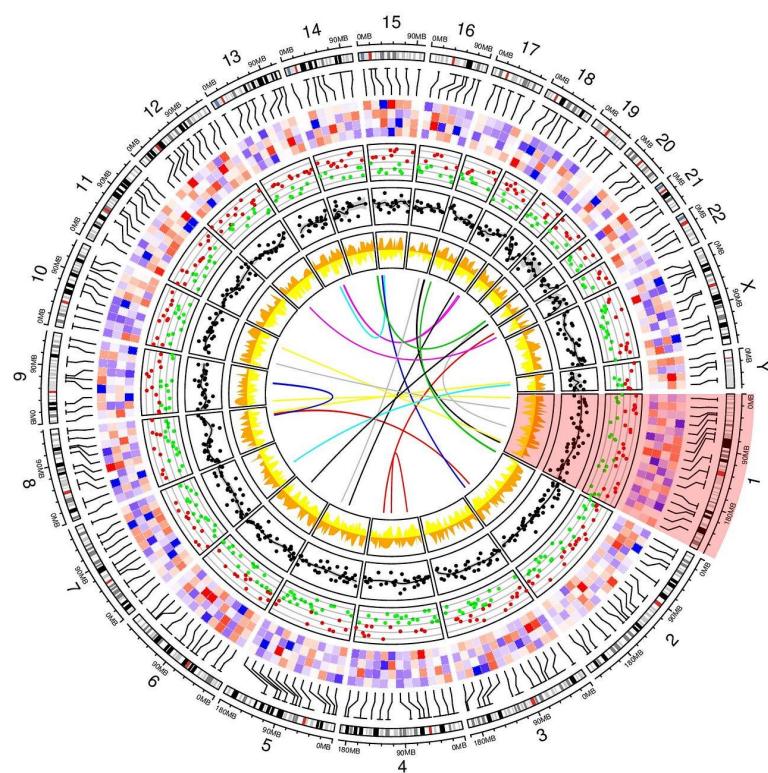
Shown: cancer biology, but many fields similarly full of junk



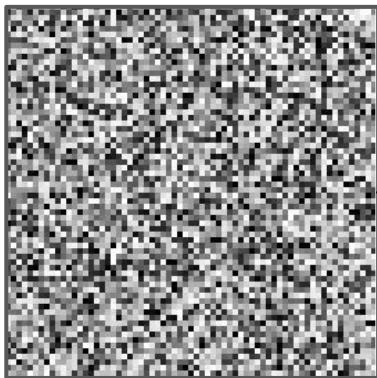
Reproducibility in Cancer Biology: Challenges for assessing replicability in preclinical cancer biology

...and if bench science has a generalizability crisis,
wait til you meet its *in silico* cousins...

- There's often nothing to reproduce other than specific figures on specific data used in the paper
 - ...despite making biological claims which sound general...
- “Code available upon request”



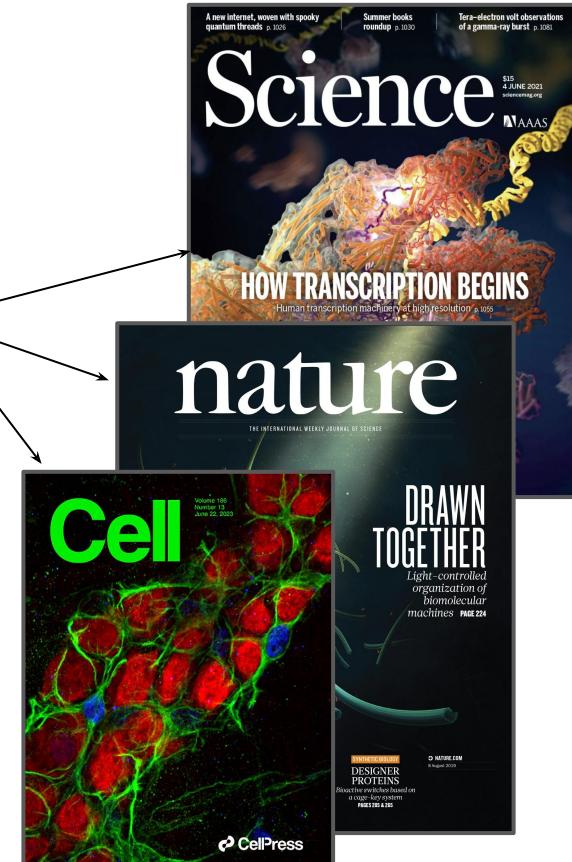
What is this class even about?



Biological
Noise

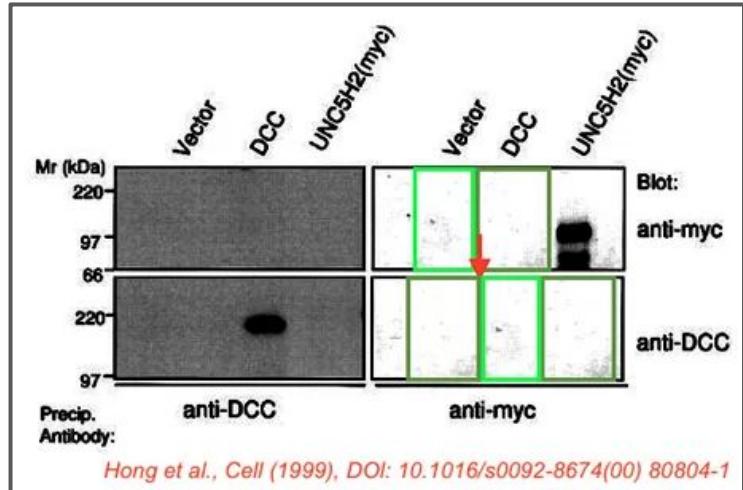


"statistically significant"
"predictive model"
"machine learning"



What is this class not about?

- BCB 731 is (mostly) not about:
 - intentional fraud
 - research “misconduct”
 - (in a narrow sense)
 - data fabrication
 - data manipulation / editing
- We will look at a few examples of intentional fraud but **unclear that authors of bad papers know they’re doing anything wrong**
 - probably trained to find & rewarded for high impact findings

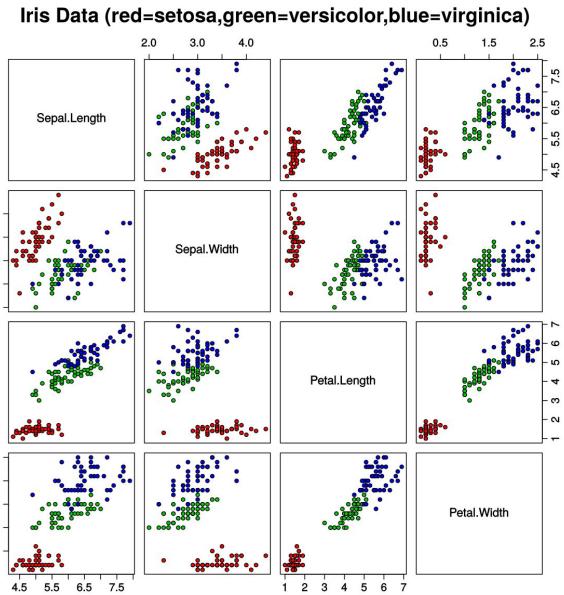


So, how do mostly honest researchers end up publishing so much junk?

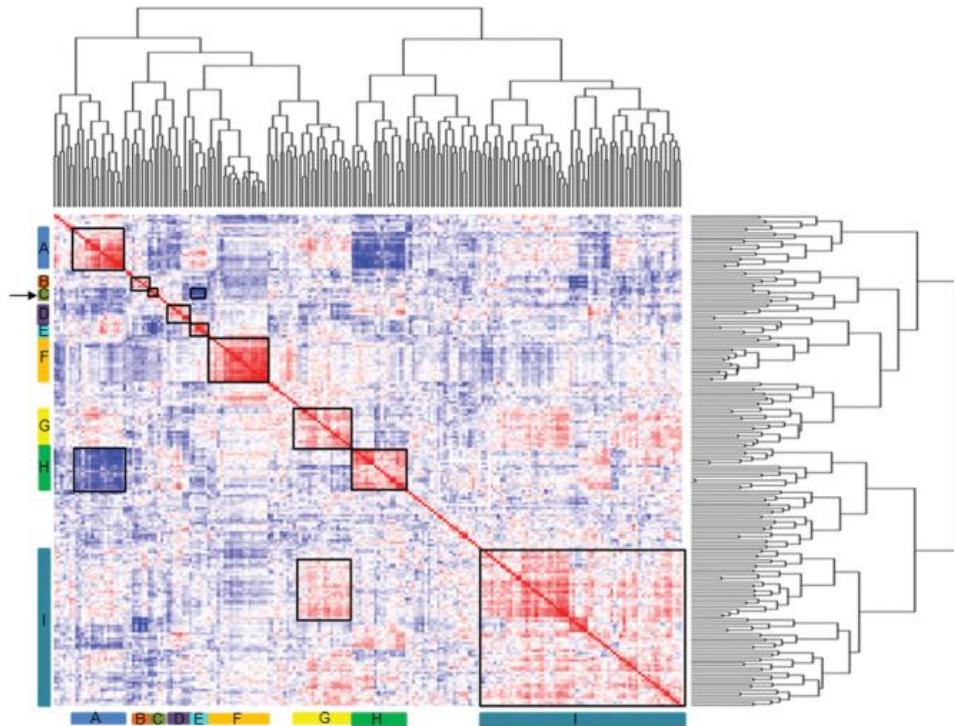
- **High throughput biological assays**
 - n (number of samples) $\ll p$ (number of features)
- **“Garden of forking paths” in data analysis**
 - Researcher Degrees of Freedom
- **Unintentionally doing machine learning**
 - Stats curriculum often doesn’t prepare for dataset (construction, preprocessing, featurization) or evaluating predictive models

The curse of dimensionality

Classical statistics was
designed for data like this:



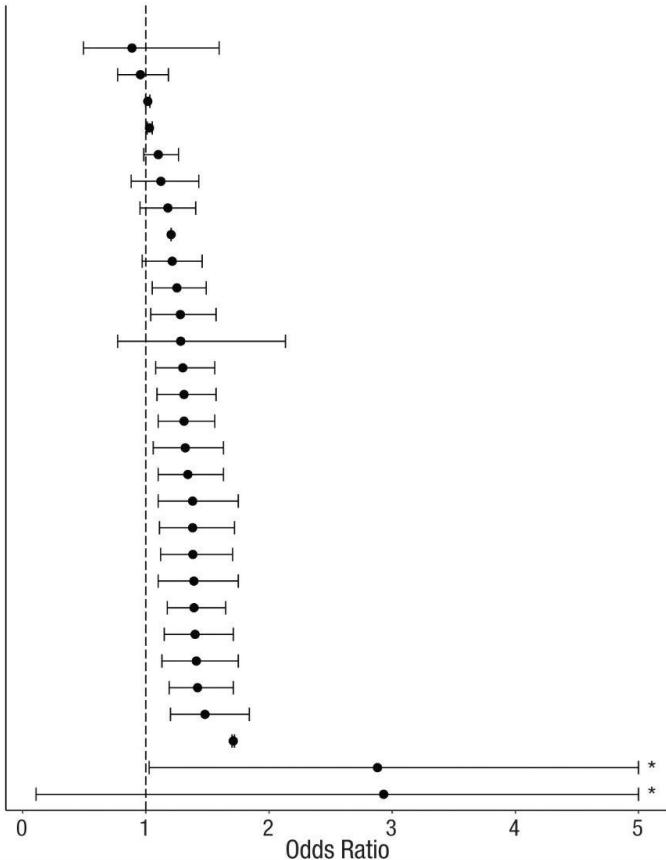
Our data looks like this:



Researcher Degrees of Freedom

Many Analysts,
One Data Set:
Making
Transparent How
Variations in
Analytic Choices
Affect Results

Analytic Approach	Odds Ratio
Zero-Inflated Poisson Regression	0.89
Bayesian Logistic Regression	0.96
Hierarchical Log-Linear Modeling	1.02
Multilevel Regression and Logistic Regression	1.03
Hierarchical Bayes Model	1.10
Logistic Regression	1.12
OLS Regression With Robust Standard Errors, Logistic Regression	1.18
Spearman Correlation	1.21
WLS Regression With Clustered Standard Errors	1.21
Multiple Linear Regression	1.25
Clustered Robust Binomial Logistic Regression	1.28
Linear Probability Model	1.28
Hierarchical Generalized Linear Modeling With Poisson Sampling	1.30
Multilevel Logistic Regression Using Bayesian Inference	1.31
Mixed-Model Logistic Regression	1.31
Hierarchical Poisson Regression	1.32
Linear Probability Model, Logistic Regression	1.34
Generalized Linear Mixed Models	1.38
Multilevel Logistic Regression	1.38
Mixed-Effects Logistic Regression	1.38
Generalized Linear Models for Binary Data	1.39
Negative Binomial Regression With a Log Link	1.39
Cross-Classified Multilevel Negative Binomial Model	1.40
Poisson Multilevel Modeling	1.41
Multilevel Logistic Binomial Regression	1.42
Generalized Linear Mixed-Effects Models With a Logit Link	1.48
Dirichlet-Process Bayesian Clustering	1.71
Tobit Regression	2.88
Poisson Regression	2.93



Objective

- Learn the red flags!
 - “Huh, seems like they might be training and testing their predictor on the same data...”
 - “How did they get $p < 10^{-200}$ on 23 samples?”
 - “Looks like their model uses test samples as part of feature selection...”
 - “They don’t say why they chose *that* particular gene set, I wonder if they also looked at other ones...”

Class format: role playing

- Every week we'll do a close read of a paper I think has at least one crucial problem
 - I might be wrong! Maybe the analyses are all great, we'll figure that out together.
- Small groups present each paper from two different roles
 - **Optimist:** gullible
 - **Critic:** highlight the core “trick” or misapplied method that helped support a non-generalizable claim

 *Fin*