# The DAMP Hypothesis: Unscheduled Cell Death Regulates the Immune Tumor Microenvironment
## University of Pittsburgh – Center for DAMP Biology

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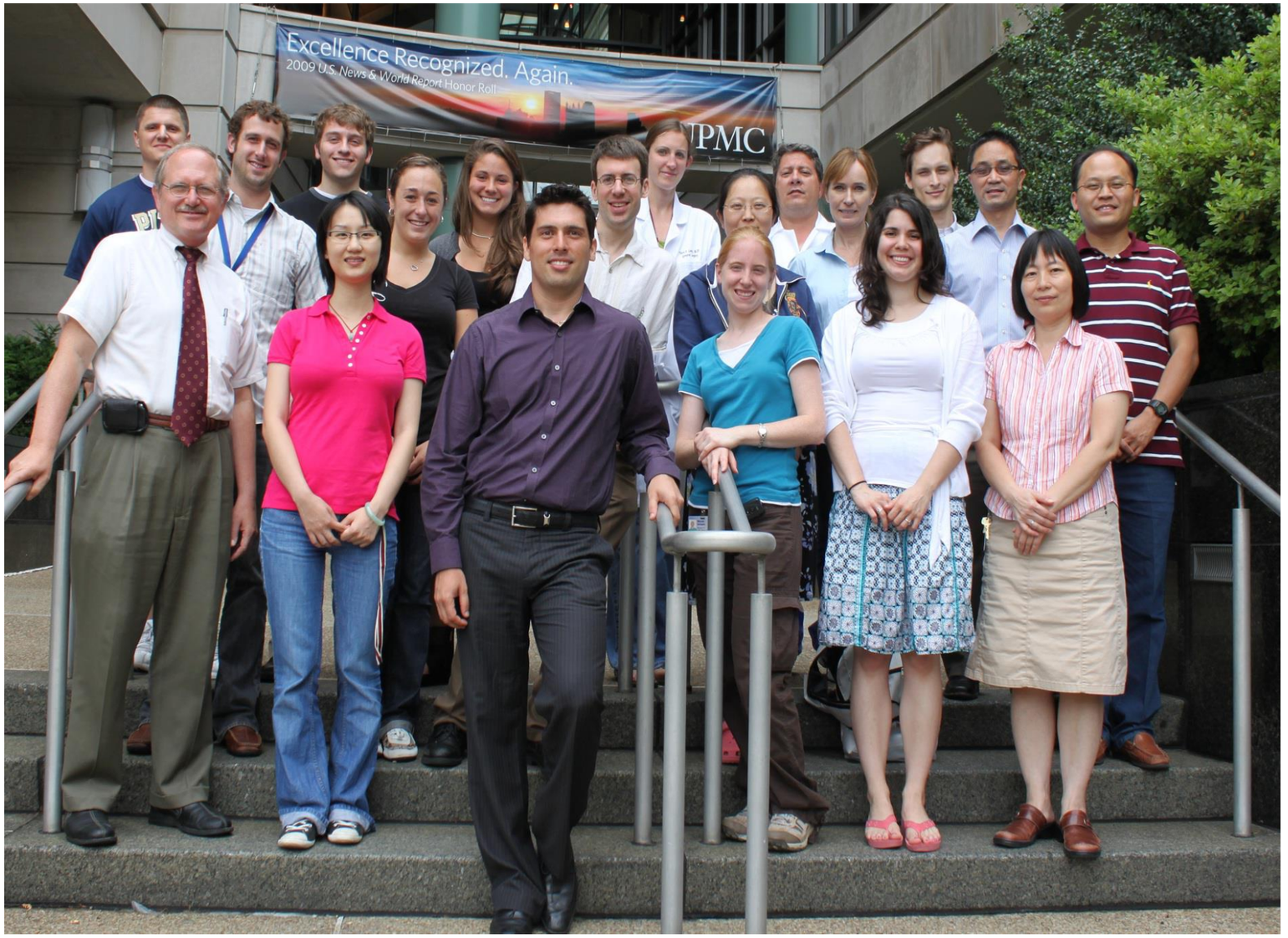
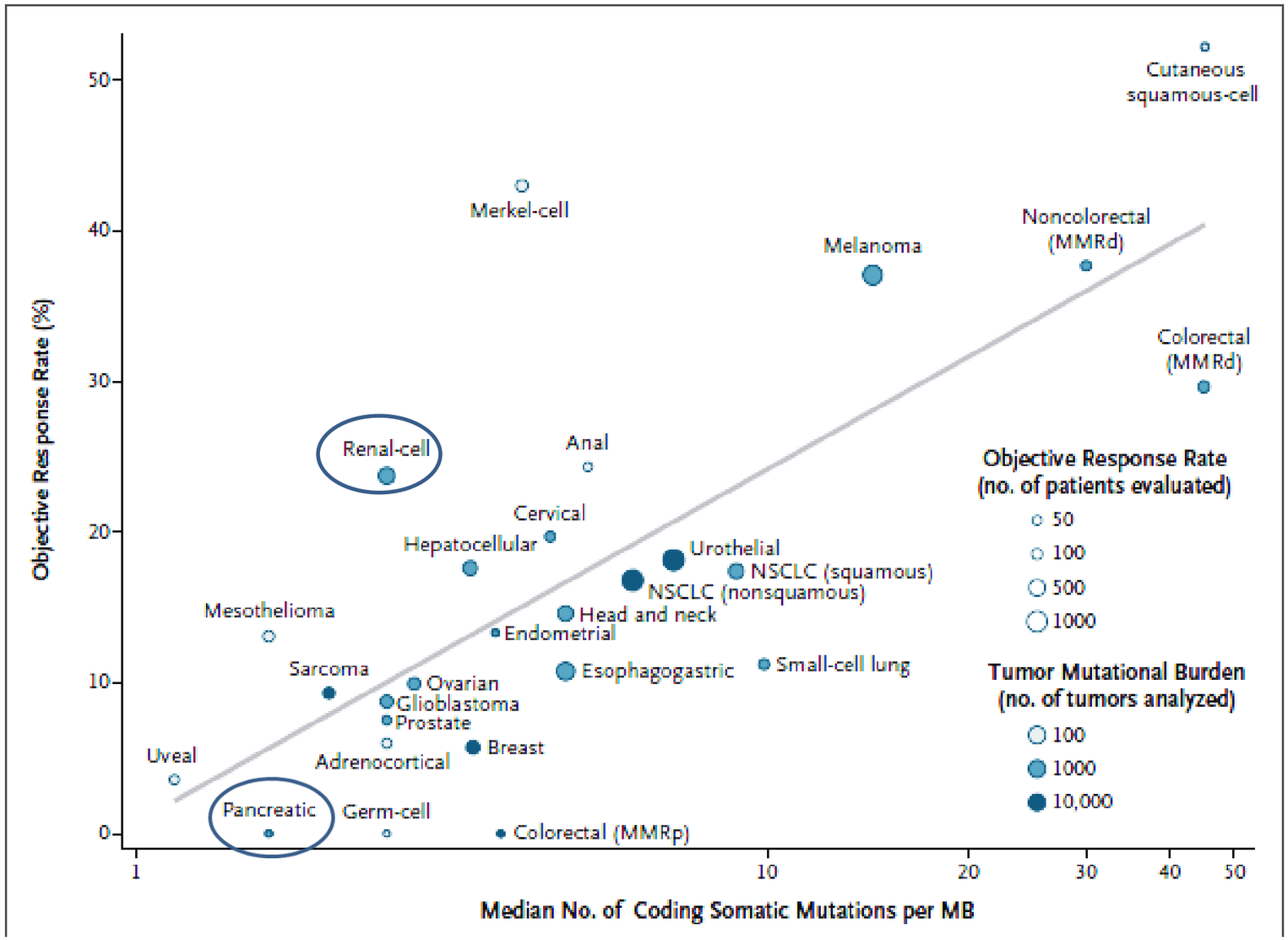
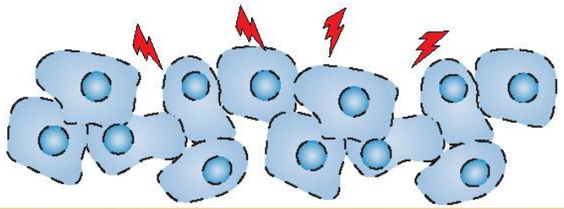


# Correlation between Tumor Mutational Burden and Objective Response Rate with Anti–PD-1 or Anti–PDL1 Therapy in 27 Tumor Types.

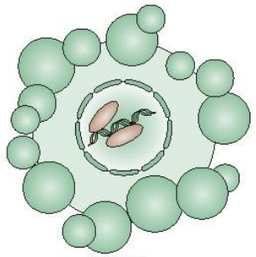
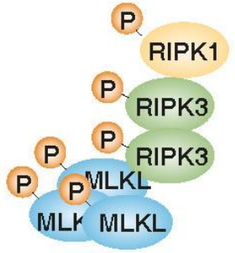
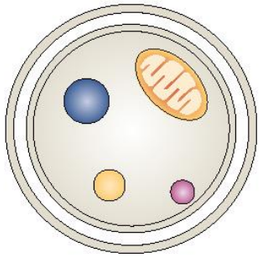
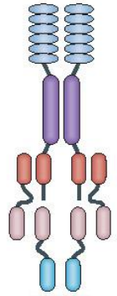
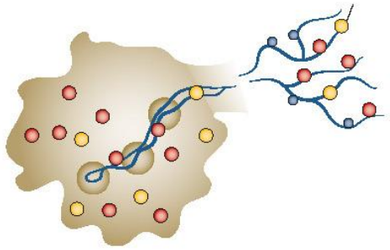
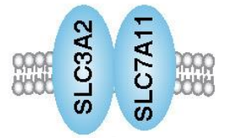



n engl j med 377;2
nejm.org Decembe
21, 2017

1) Autophagy 101
2) The DAMP Hypothesis
3) Pancreatic Cancer
4) Renal Cancer
5) Biomarkers of the DAMP Hypothesis



Dead, dying, or injured cells

| Apoptotic body | Necrosome | Autophagosome | Inflammasome | NETs | System $X_c^-$ |
|---|---|---|---|---|---|
|  |  |  |  |  |  Lipid peroxdation |
| Apoptosis | Necroptosis | Autophagy | Pyroptosis | NETosis | Ferroptosis |
| DNA, Histone | HMGB1, ATP | HMGB1, ATP | IL1, IL18, HMGB1, K+ | DNA, HMGB1, Histone | HMGB1, PUFA |

Toll-like receptors, NOD-like receptors, RIG-I-like receptors, AIM2-like receptors, and RAGE

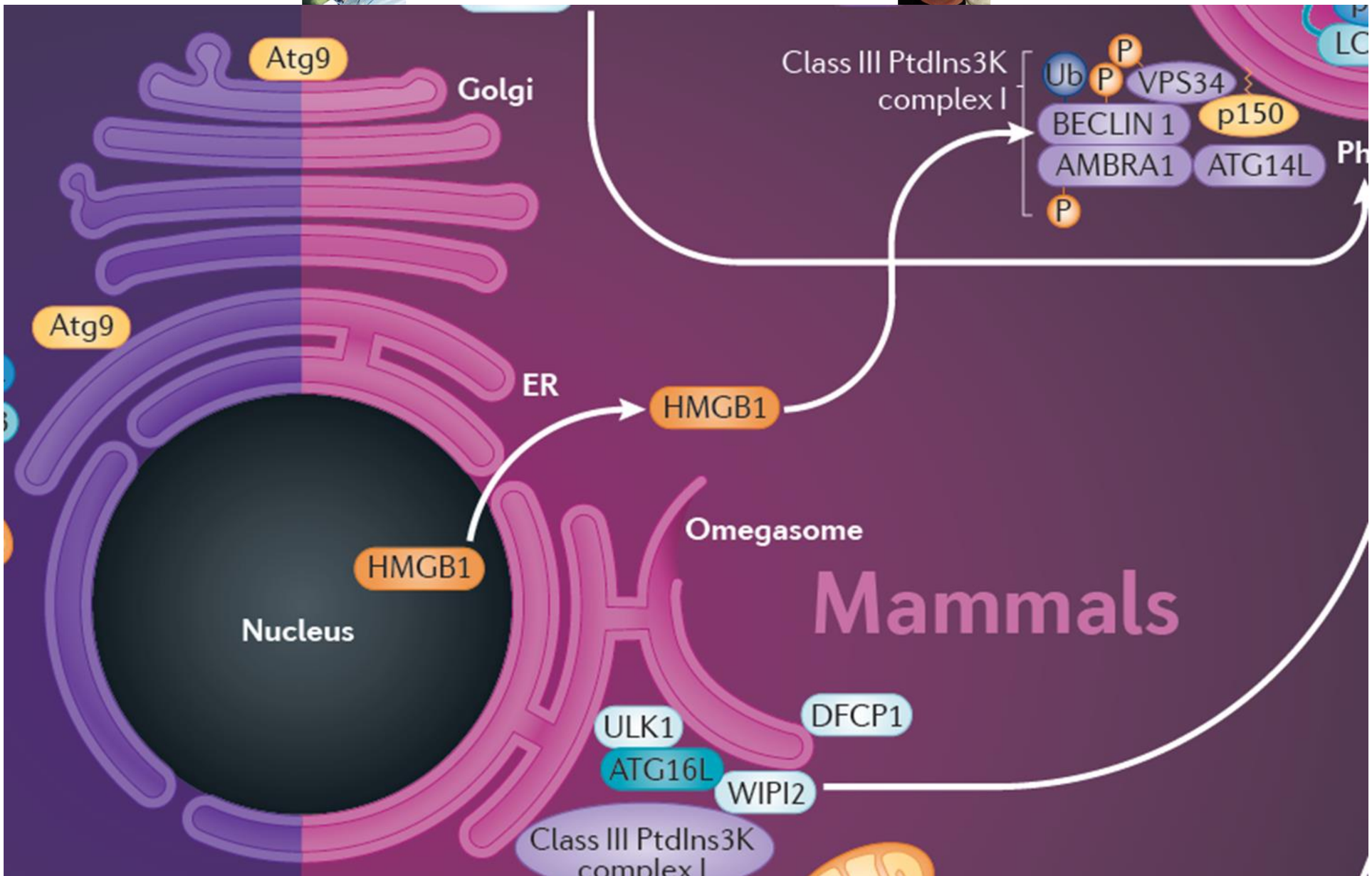
Regulation of inflammation, immune, metabolism and stress response

**Yoshinori Ohsumi**
**Autophagy**
**Nobel Prize 2016**

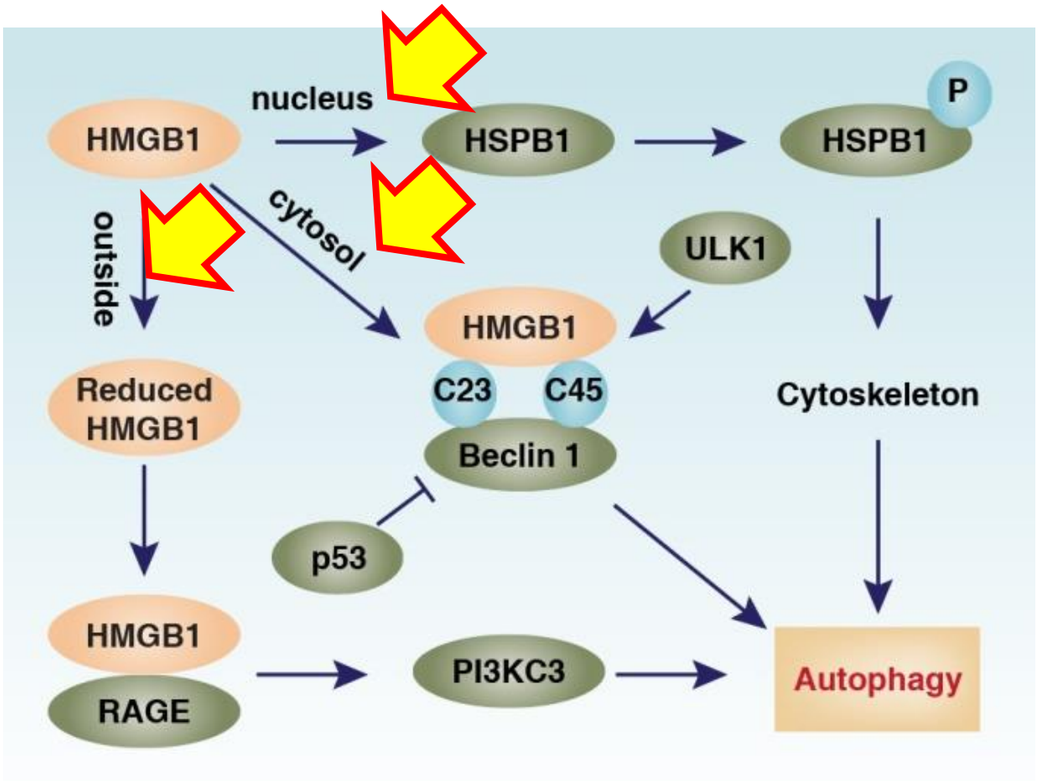
Japanese scientist Yoshinori Ohsumi was awarded this year's Nobel Prize in medicine on Monday for discoveries related to the degrading and recycling of cellular components. The Karolinska Institute honored Ohsumi for "brilliant experiments" in the 1990s on autophagy, the machinery with which cells recycle their content. Disrupted autophagy has been linked to various diseases, including Parkinson's, diabetes and cancer, the institute said. Ohsumi was born in 1945 in Fukuoka, Japan. He is currently a professor at the Tokyo Institute of Technology.

# Autophagy: molecular mechanisms and disease outcomes

Daniel J. Klionsky and Vojo Deretic



# HMGB1-dependent Autophagy




Tang *et al, **J Cell Biol***. 2010
Tang *et al* ***Cell Metab***. 2011

Tang *et al, **Oncogene*** 2010
Huang et al, ***Cancer Res.*** 2012

Livesey et al, ***Cancer Res.*** 2012
Yang *et al, **Autophagy***. 2015

The NEW ENGLAND JOURNAL of MEDICINE

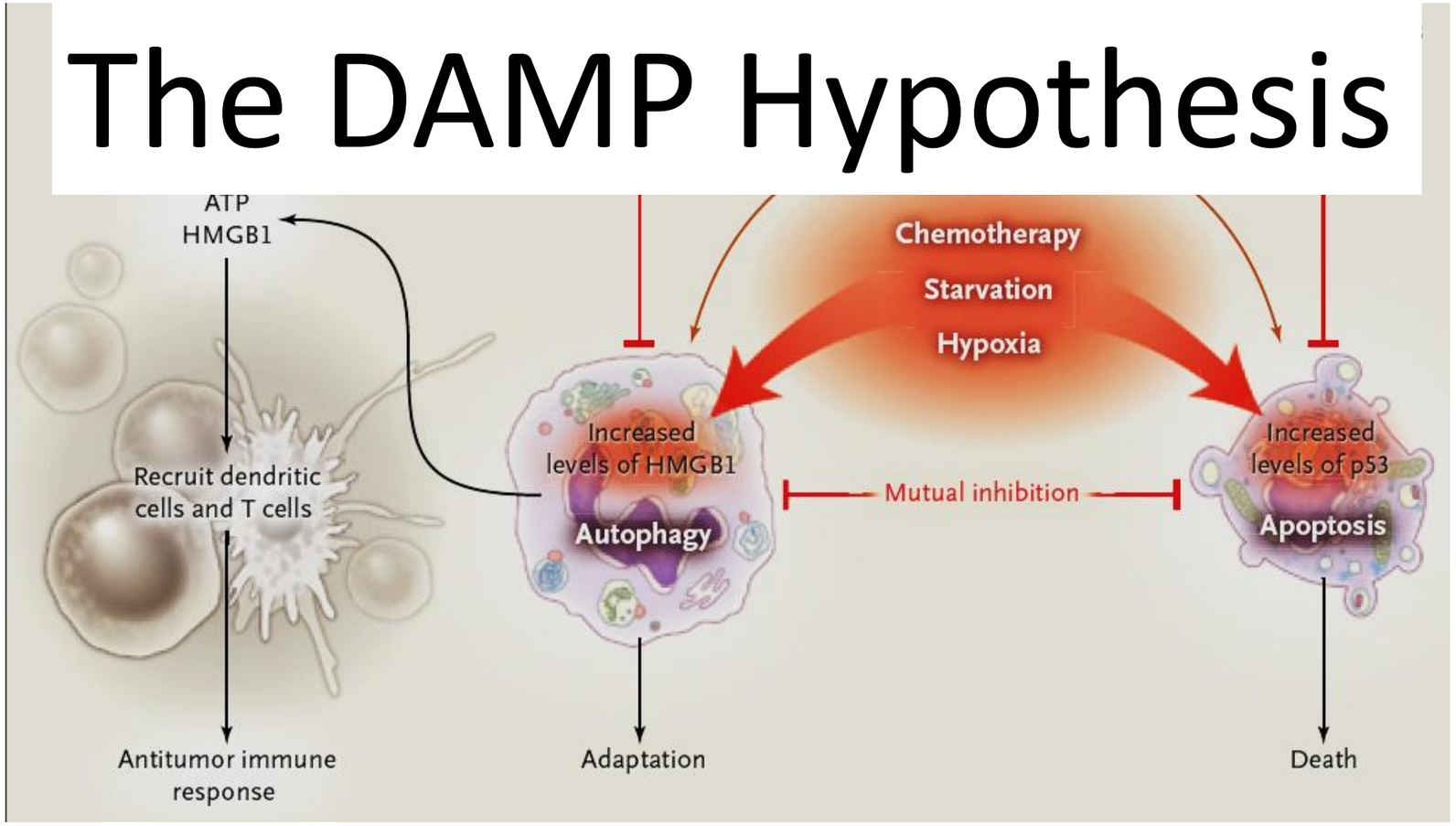
366:1156-1158; March 22, 2012

CLINICAL IMPLICATIONS OF BASIC RESEARCH

## Tumor-Cell Death, Autophagy, and Immunity

Louis M. Weiner, M.D., and Michael T. Lotze, M.D.

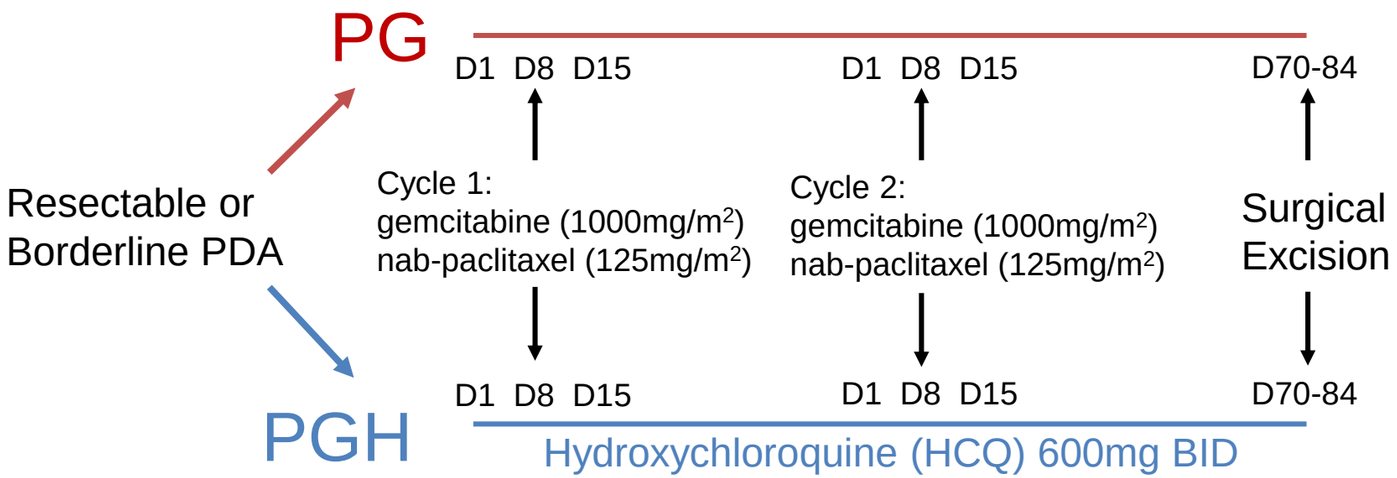
# The DAMP Hypothesis



# Background: Hydroxychloroquine

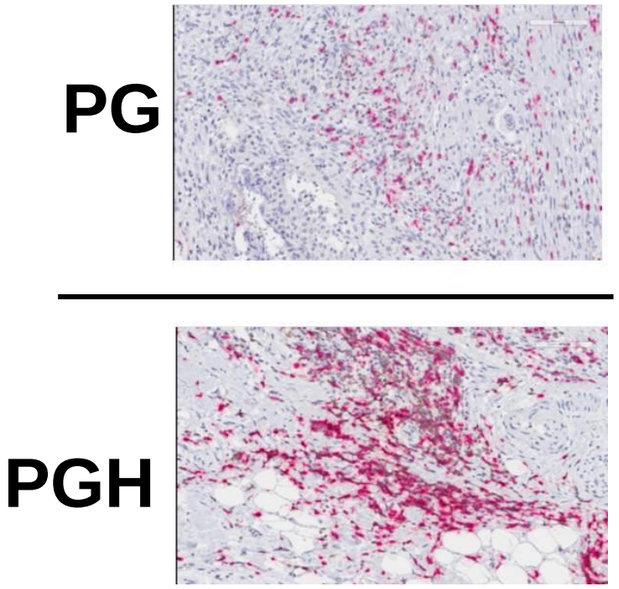
- **Anti-malarial drug**
- **Useful in SLE for cutaneous, MSK disease and fatigue; very safe and long track record**
- **Mild to moderate disease-modifying properties in RA**
- **Inhibits Autophagy by Preventing Lysosomal Fusion**

# UPCI 13-074 Neoadjuvant Pancreatic Cancer Trial Design

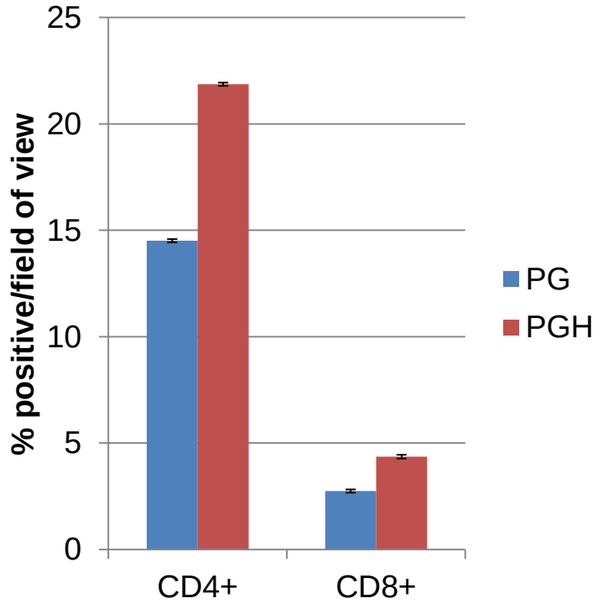



(R01 CA160417-01A1)

# Enhanced T cell Infiltration in PGH Paclitaxel, Gemcitabine, Hydroxychloroquine

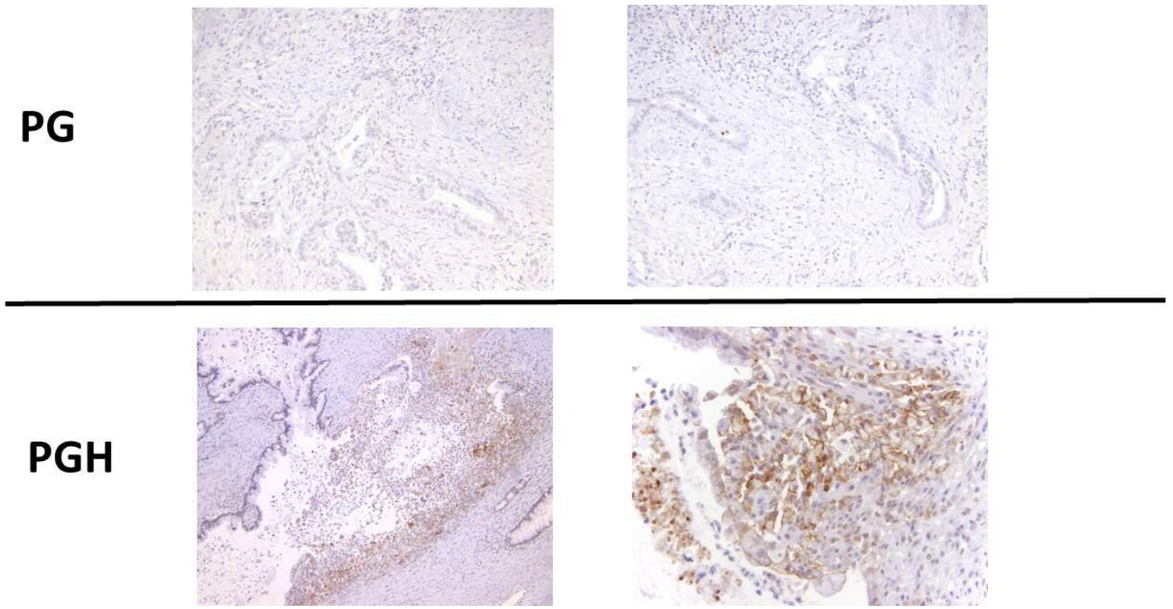



Pink: CD4; Brown: CD8



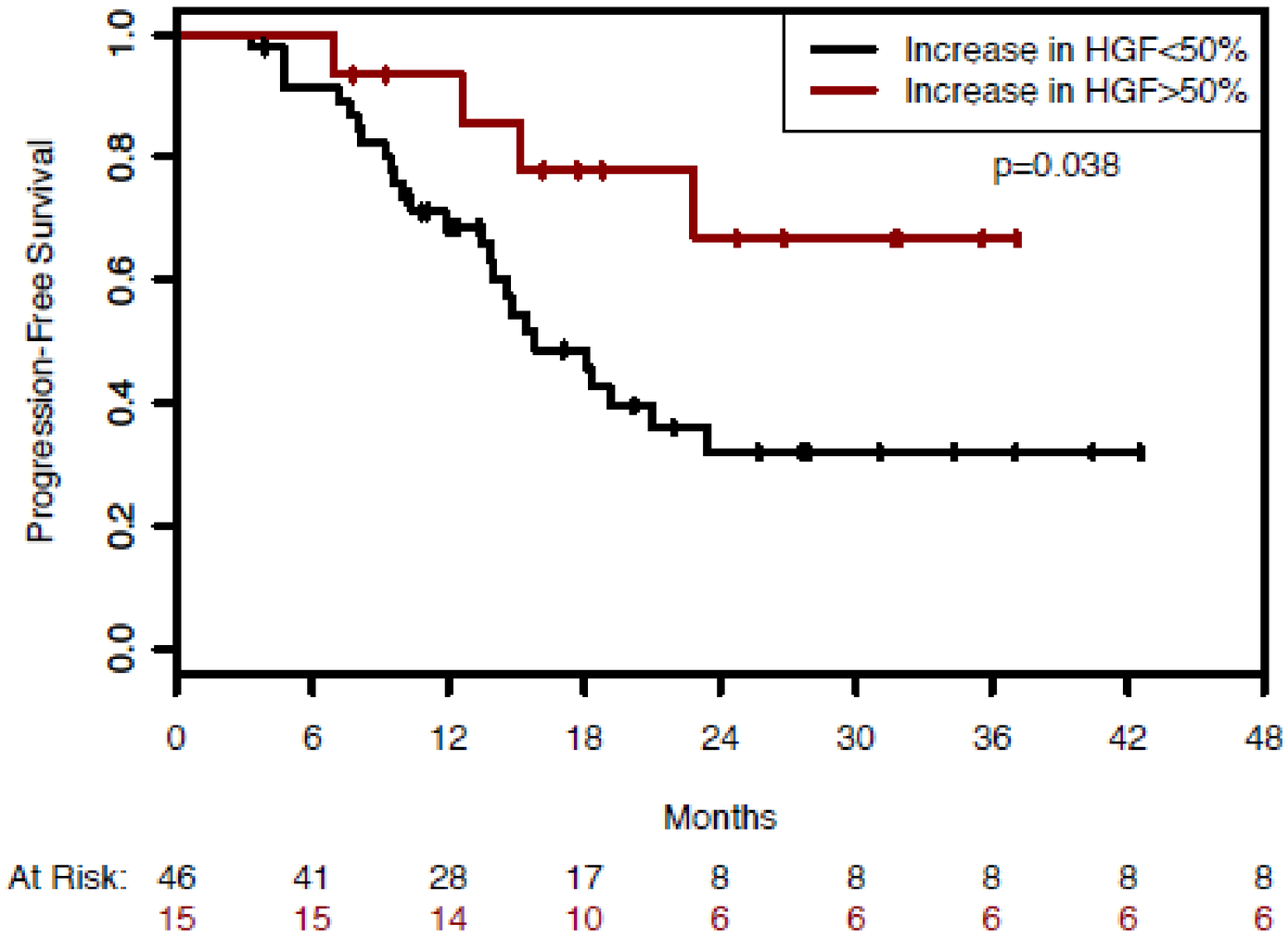

*p=0.016 *p=0.046

# Increased PD-L1 Expression in PGH



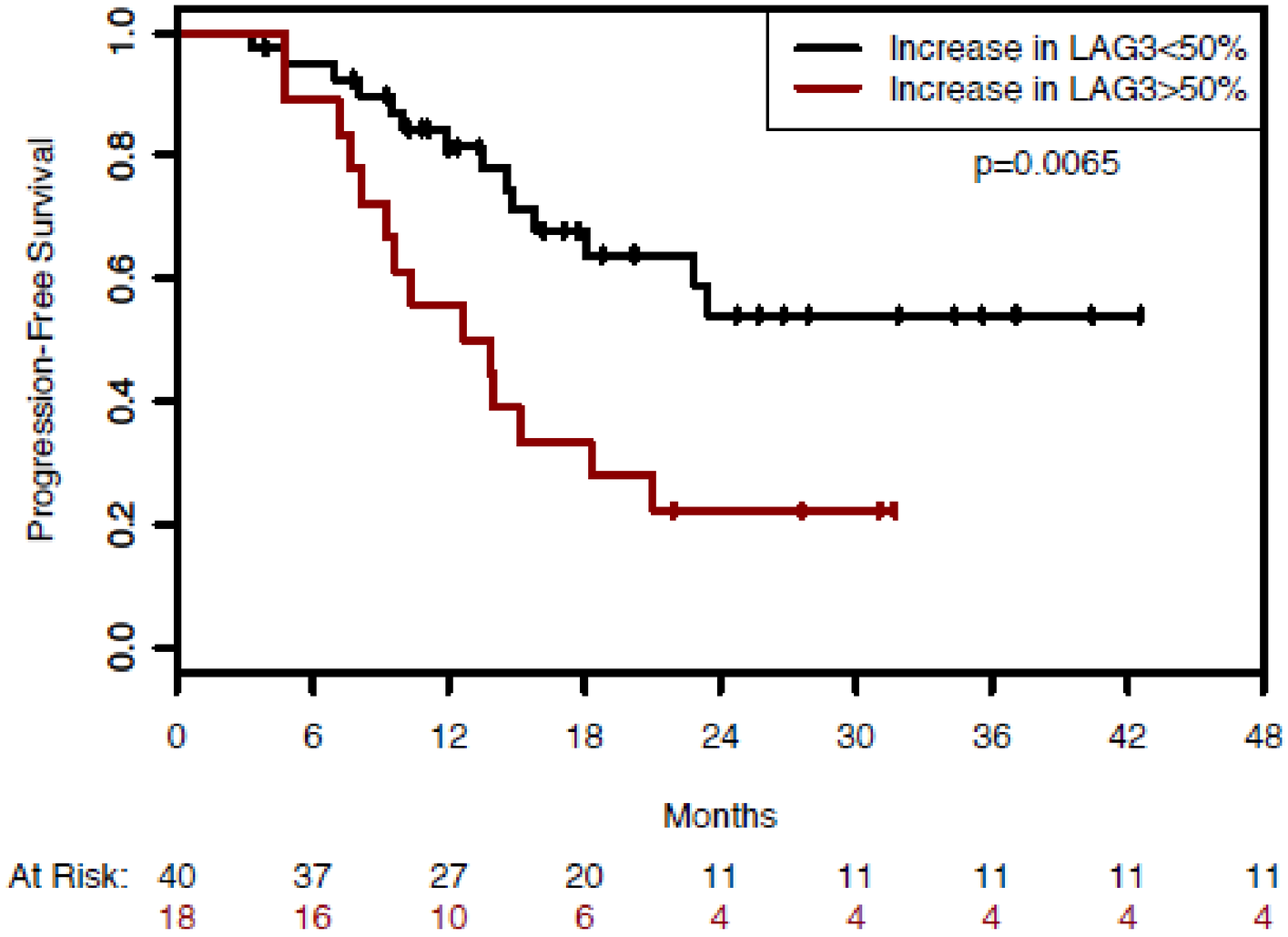

Brown: programmed death ligand I (PD-L1)

# Increased HGF with Rx Better



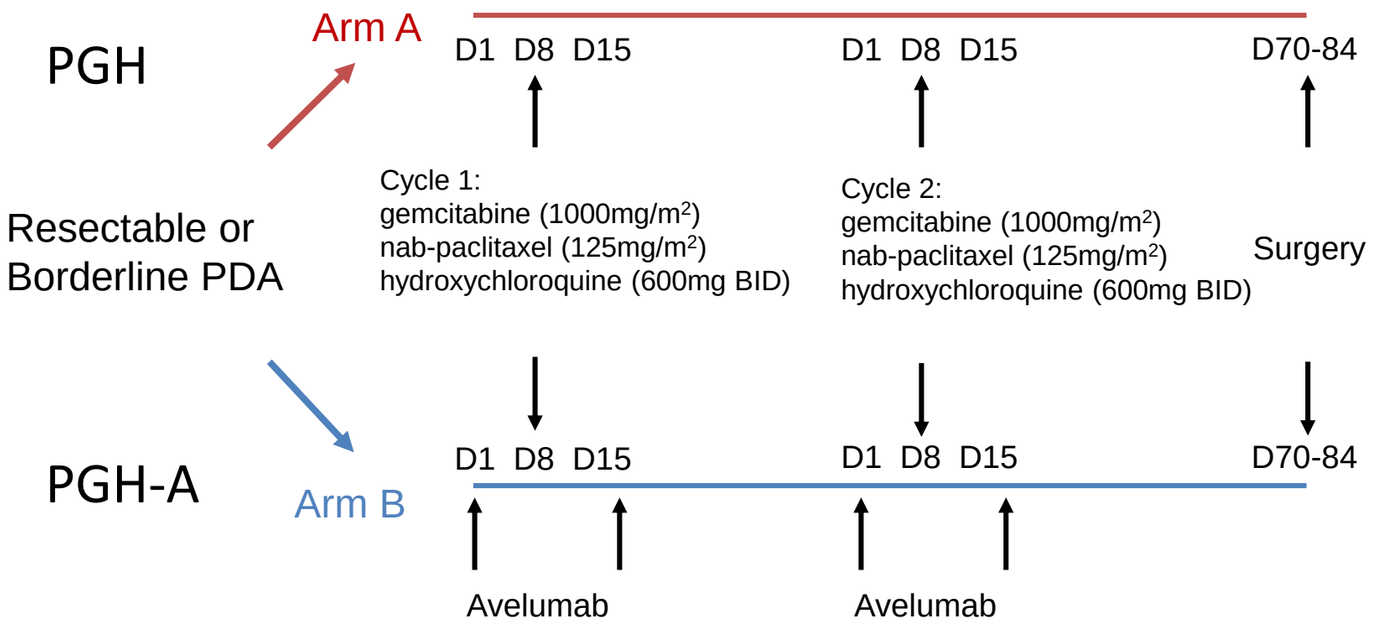

| | Median | Lower 95% CI | Upper 95% CI |
|---|---|---|---|
| 1 | 21.45 | 2.19 | 15.83 |
| 2 | 31.70 | 3.39 | NA |

# Increased sLAG3 with Rx Worse




| | Median | Lower 95% CI | Upper 95% CI |
|---|---|---|---|
| 1 | 26.48 | 2.19 | NA |
| 2 | 16.95 | 2.72 | 13.26 |

# Current Trial Design-+/- Ab PDL1



# Conclusions 1: Autophagy Inhibition in Pancreatic Cancer

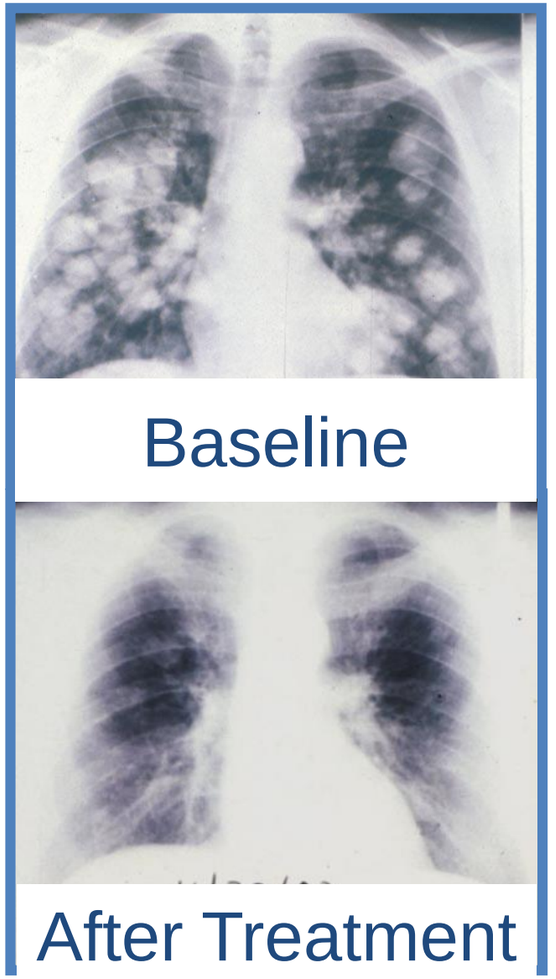
- **Autophagy inhibition + chemotherapy improves:**
  - histopathologic response:
    - Evans >50% destruction: n=24 vs n=12, p=0.0095
  - biochemical response:
    - % change CA 19-9, mean 1189 vs 368; p=0.0095
- **Corollary studies suggest:**
  - diminished stromal activation
  - increased immune infiltration (CD4 and CD8)
  - increased PD-L1

# The First Cellular (Treg) Checkpoint Inhibitor - High Dose IL-2

- High-dose IL-2 (HDIL2) is an FDA-approved immunotherapy for patients with renal cell carcinoma (RCC) and melanoma.

- The mechanism is related to T and NK cell activation and profligate cytokine production.

- HDIL-2 toxicity
  - ~65% of patients have problems with high toxicity
  - Development of cytokine storm
  -Co-administration of iNOS inhibitors, soluble TNF-R or IL-1R has yielded only modest reduction of serious side effects




- IL-2 treatment may induce a 'systemic cytokine-induced autophagic syndrome' and temporally limited tissue dysfunction. --- *AR. Chavez, X Liang, MT Lotze. Ann.N.Y.Acad.Sci.1182:14-27 (2009)*

# New Cytokine and Checkpoints In the Clinic

| Company | Agent | Checkpoint inhibitor combination partner(s) | Indication | Stage |
|---|---|---|---|---|
| Nektar/BMS | NKTR-214, IL-2Rβ (CD122)-biased PEGylated IL-2 | Opdivo, Yervoy, Keytruda | Solid tumors | Phase 2 ongoing<br>Phase 3 to start mid-2018 |
| ARMO Biosciences | AM0010 (pegilodecekin), PEGylated recombinant human IL-10 | Keytruda, Opdivo | NSCLC | Phase 2 |
| Roche (Basel, Switzerland) | RO6874281 (RG7461), engineered IL-2 variant conjugated to anti-FAP antibody | Tecentriq (atezolizumab) | Solid tumors | Phase 2 |
| Altor Bioscience (NantCell) | ALT-803, IL-15 superagonist | Opdivo | NSCLC | Phase 1/2 |
| Merck KGaA (Darmstadt, Germany) | NHS-IL12, IL-12 fused to IgG1 antibody targeted to necrotic regions | Bavencio (avelumab) | Solid tumors | Phase 1 |
| Novartis (Basel, Switzerland) | NIZ985 (IL-15/sIL-15rα), heterodimeric human IL-15 | PDR001 | Metastatic solid tumors | Phase 1 |
| US National Cancer Institute | Recombinant human IL-15 | Opdivo, Yervoy | Metastatic solid tumors | Phase 1 |
| Alkermes (Dublin) | ALKS 4230, fusion protein of circularly permuted IL-2 & IL-2Rα | None yet | Refractory solid tumors | Phase 1 |
| Medicenna | MDNA-109, IL-2 superagonist | NA | NA | IND 2019 |
| Nektar | NKTR-255, IL-15 receptor agonist | NA | NA | IND 2019 |

NSCLC, non-small cell lung cancer; NA, not applicable; IND, new drug application.

**NATURE BIOTECHNOLOGY** VOLUME 36 NUMBER 5 M

# Renal Cell Carcinoma: Approved Agents 2018 (VHL/HIF1α)


**VEGFR TKI**

**VEGFR/MET TKI**

**mTOR inhibitor**

**Immune therapy**

**Neutralizing anti-VEGF mAb**

**ACS estimates RCC US 2018:**

- 63,990 cases (40,610 men; 23,380 women)
- 14,400 people (9,470 men and 4,930 women) will die

# The Strange Immunobiology of RCC

| RESPONSE | INTEFERON α | IL- 2 | CTLA4 AB | PD-1 AB | TIL |
|---|---|---|---|---|---|
| MELANOMA | + | +++ | ++ | ++ | ++++ |
| RCC | + | +++ | ++ | ++ | - |


1: **Lotze MT**, Maranchie J, Appleman L. Inhibiting autophagy: a novel approach for the treatment of renal cell carcinoma. Cancer J. 2013 Jul-Aug;19(4):341-7

2: Romo de Vivar Chavez A, de Vera ME, Liang X, **Lotze MT**. The biology of IL-2 efficacy in the treatment of patients with RCC. Med Oncol. 2009; 1:3-12.

3: Bernhard H, Maeurer MJ, Jäger E, Wölfel T, Schneider J, Karbach J, Seliger B, Huber C, Storkus WS, **Lotze MT**, Meyer zum Büschenfelde KH, Knuth A. Recognition of human RCC and melanoma by HLA-A2-restricted CTL is mediated by shared peptide epitopes and up-regulated by IFNg. Scand J Immunol. 1996;44:285-92.

4: Maeurer MJ, Martin DM, Castelli C, Elder E, Leder G, Storkus WJ, **Lotze MT**. Host immune response in RCC: IL-4 and IL-10 mRNA are frequently detected in freshly collected TIL. Cancer Immunol Immunother. 1995 Aug;41(2):111-21.

5: Spencer WF, Linehan WM, Walther MM, Haas GP, **Lotze MT**, Topalian SL, Yang JC, Merino MJ, Lange JR, Pockaj BA, et al. Immunotherapy with IL2 and IFNα in patients with metastatic RCC with *in situ* primary cancers. J Urol. 1992 147(1):24-30.

6: Rosenberg SA, **Lotze MT**, Muul LM, et al. Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant IL-2 to patients with metastatic cancer. N Engl J Med. 1985 Dec 5;313(23):1485-92.

Published OnlineFirst February 16, 2015; DOI: 10.1158/1078-0432.CCR-14-2926

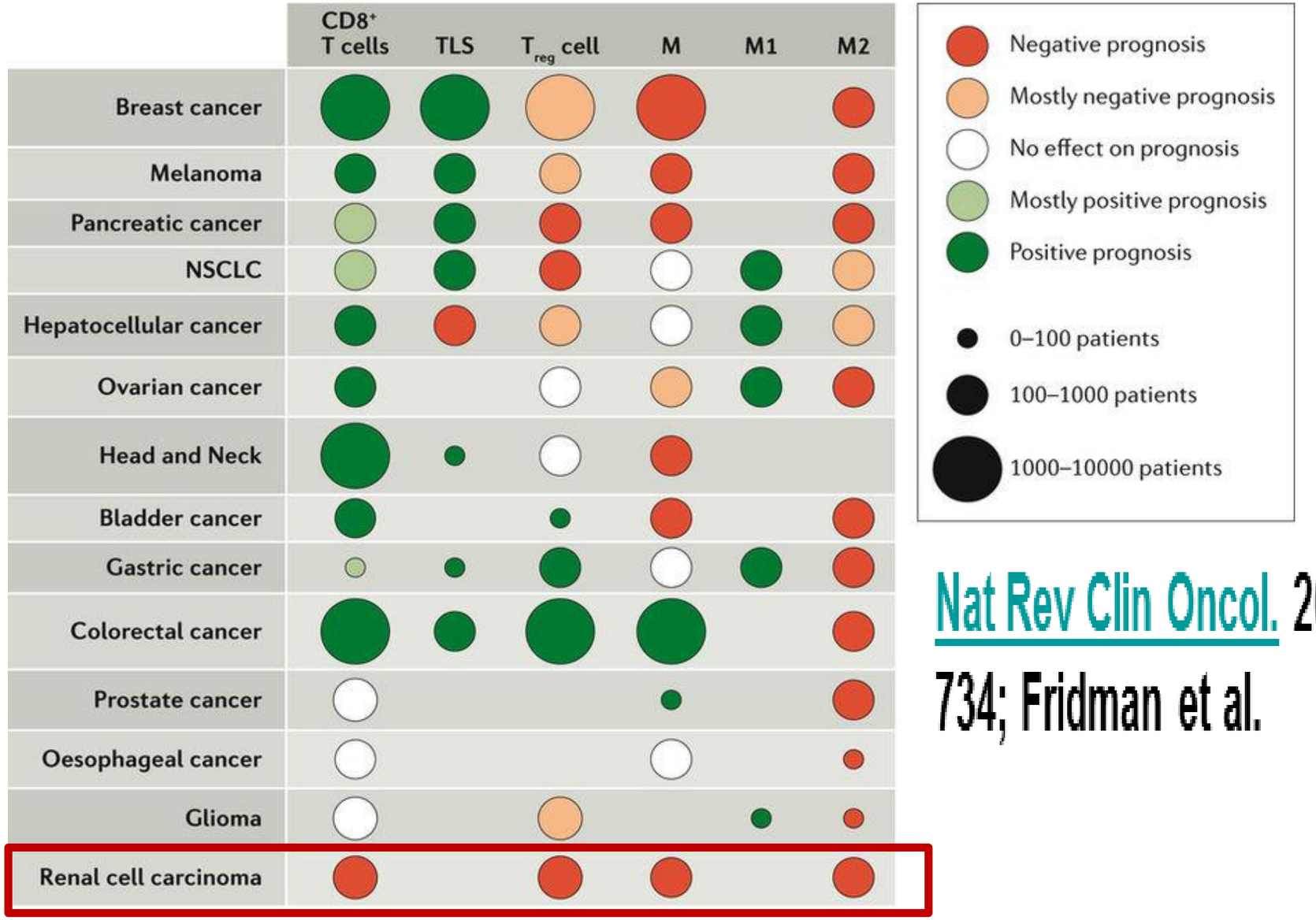


Clinical Cancer Research

…two groups of tumors with extensive $CD8^+$T-cells:

1. high expression of immune checkpoints in the absence of fully functional mature DC→↑ risk of disease progression.
2. low expression of immune checkpoints and localization of mature DC in peritumoral immune

# Unlike other Cancers, RCC Infiltration by TILs is linked to Poor Prognosis: A Matter of Immune Context?




Nat Rev Clin Oncol. 2017 Dec;14(12):717-734; Fridman et al.

*Microenvironment and Immunology*

Cancer Research

# Inhibiting Systemic Autophagy during Interleukin 2 Immunotherapy Promotes Long-term Tumor Regression

Xiaoyan Liang[1], Michael E. De Vera[1], William J. Buchser[1], Antonio Romo de Vivar Chavez[1], Patricia Loughran[1,2], Donna Beer Stolz[2], Per Basse[3], Tao Wang[4], Bennett Van Houten[4], Herbert J. Zeh III[1], and Michael T. Lotze[1,3]

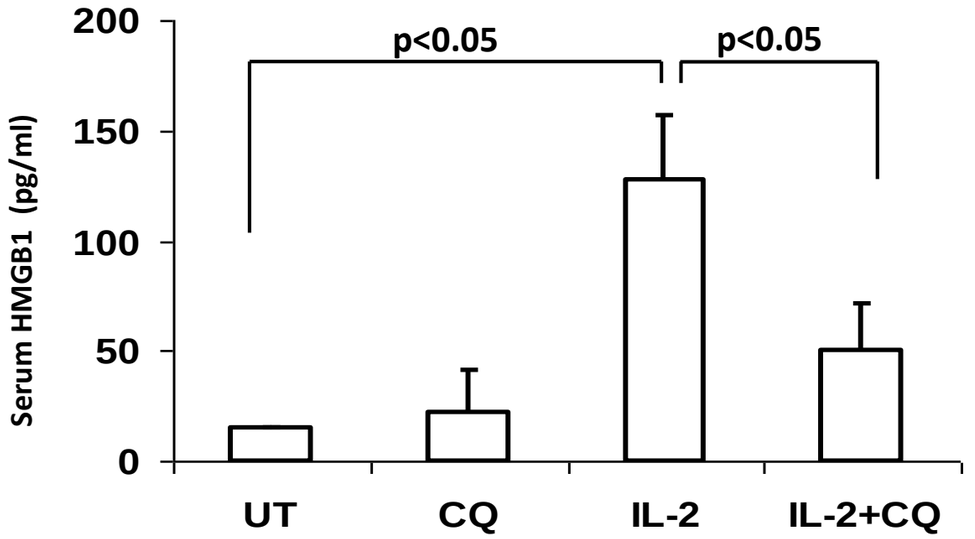
*Cancer Research 72(11): 2791~2801, 2012*

# Blocking the interleukin 2 (IL2)-induced systemic autophagic syndrome promotes profound antitumor effects and limits toxicity

Michael T. Lotze,* William J. Buchser and Xiaoyan Liang

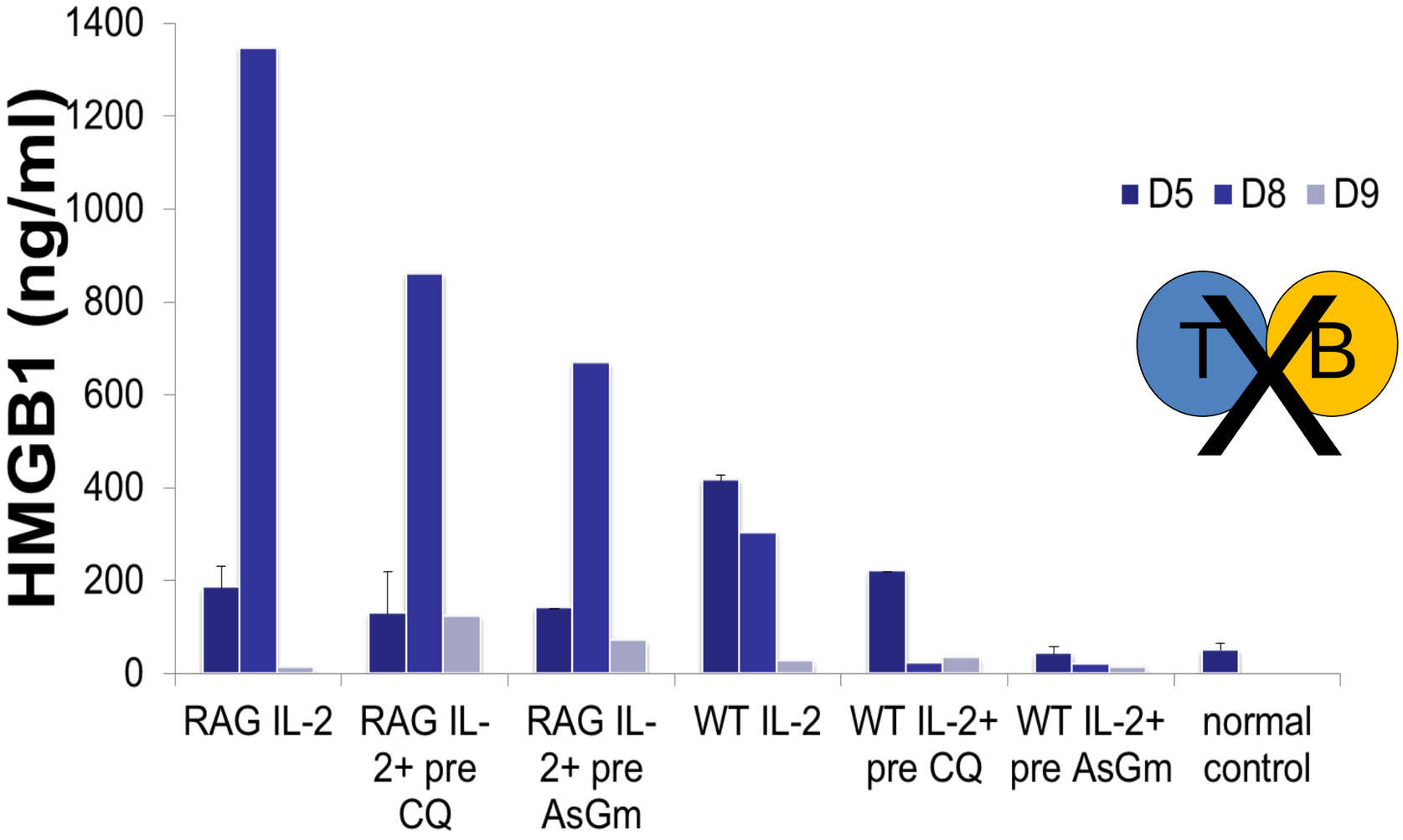
The DAMP Laboratory; Department of Surgery; Hillman Cancer Center; University of Pittsburgh Cancer Institute; University of Pittsburgh; Pittsburgh, PA USA

*Autophagy 8:8, 1264–1266; 2012;*




CQ (Chloroquine): an anti-malarial drug

# Marked Increases Serum HMGB1 RAG KO Mice Rx IL-2x5D

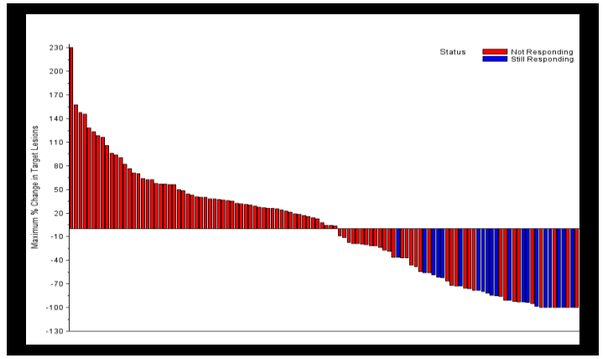


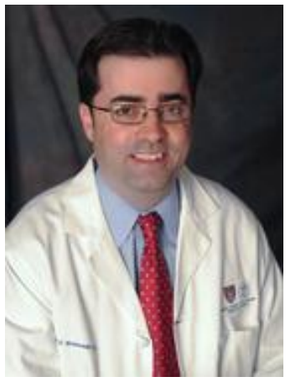

Xiaoyan Liang (梁晓燕）

Dartmouth
Harvard
Indiana
Oregon
Pittsburgh
Portland

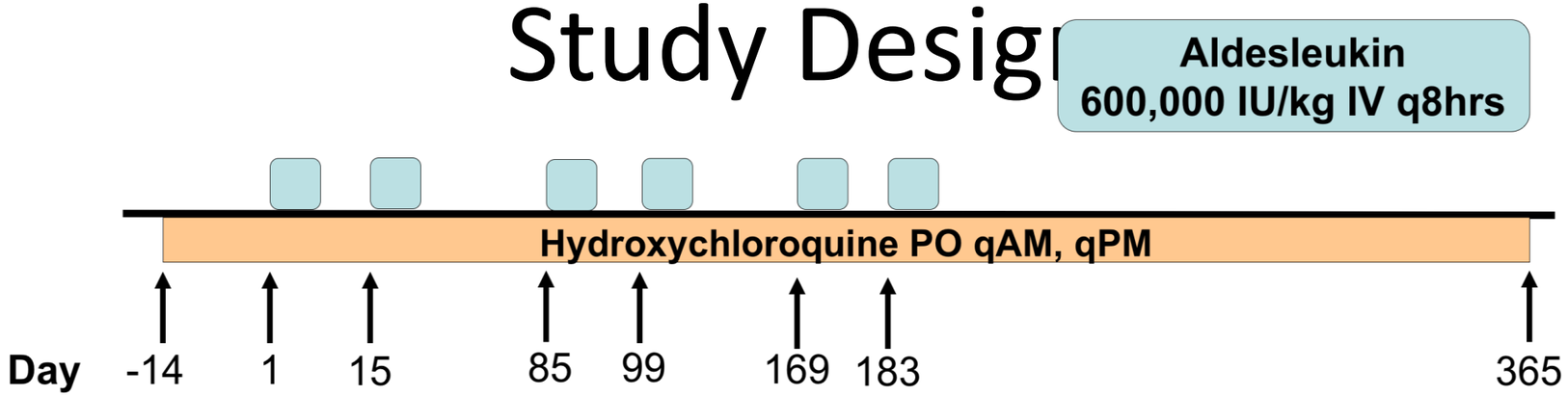
## Inhibiting the Systemic Autophagic Syndrome – A Phase I/II Study of Hydroxychloroquine and Aldesleukin in Renal Cell Carcinoma Patients (RCC) – 30 Patients

A Cytokine Working Group (CWG) Study
Principal Investigator: Michael T. Lotze, MD
Prometheus/Nestle



# Study Desig

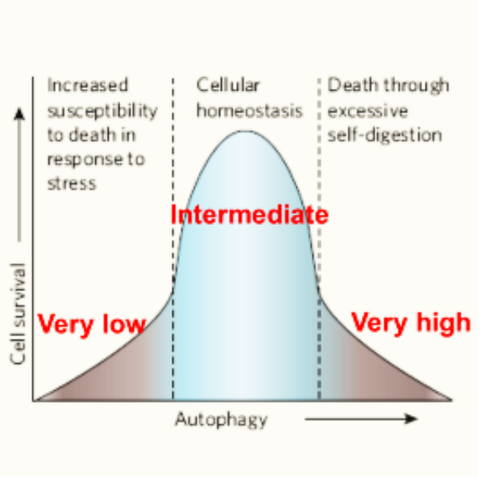



## Notable Eligibility Criteria:

- mRCC with measurable disease
- Karnofsky PS ≥ 80%
- Known G6PD deficiency exclusion
- Serum Creatinine ≤1.5 or est CrCl ≥ 60 ml/min
- Baseline QTc ≤ 470 msec
- Prior immune checkpoint inhibitor treatment allowed.

**Study Design: Single arm IL-2+ HCQ with Bayesian beta-binomial stopping rule for excessive toxicity; stopped for toxicity due to 1200mg (N=13) and remainder treated at 600mg (N=17)**

## Relationship Between The Levels Of Autophagy And Cell Death




*Nature* **446**, 745-747 (12 April 2007)

**Very low** -- The absence of autophagy increases cell death during metabolic stress and on treatment with cytotoxic chemotherapeutic agents.

**Intermediate** -- Physiological levels of autophagy are essential for normal cellular homeostasis.

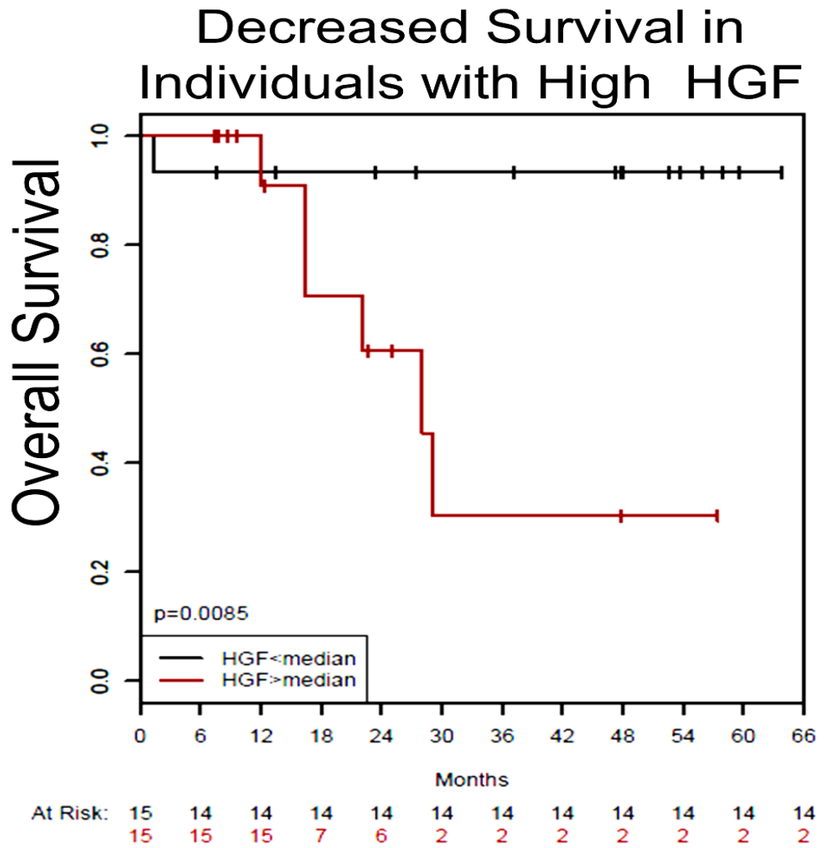
**Very high** -- excessive levels of autophagy promote cell death.

Beth Levine

# Results – 3CR, 3PR

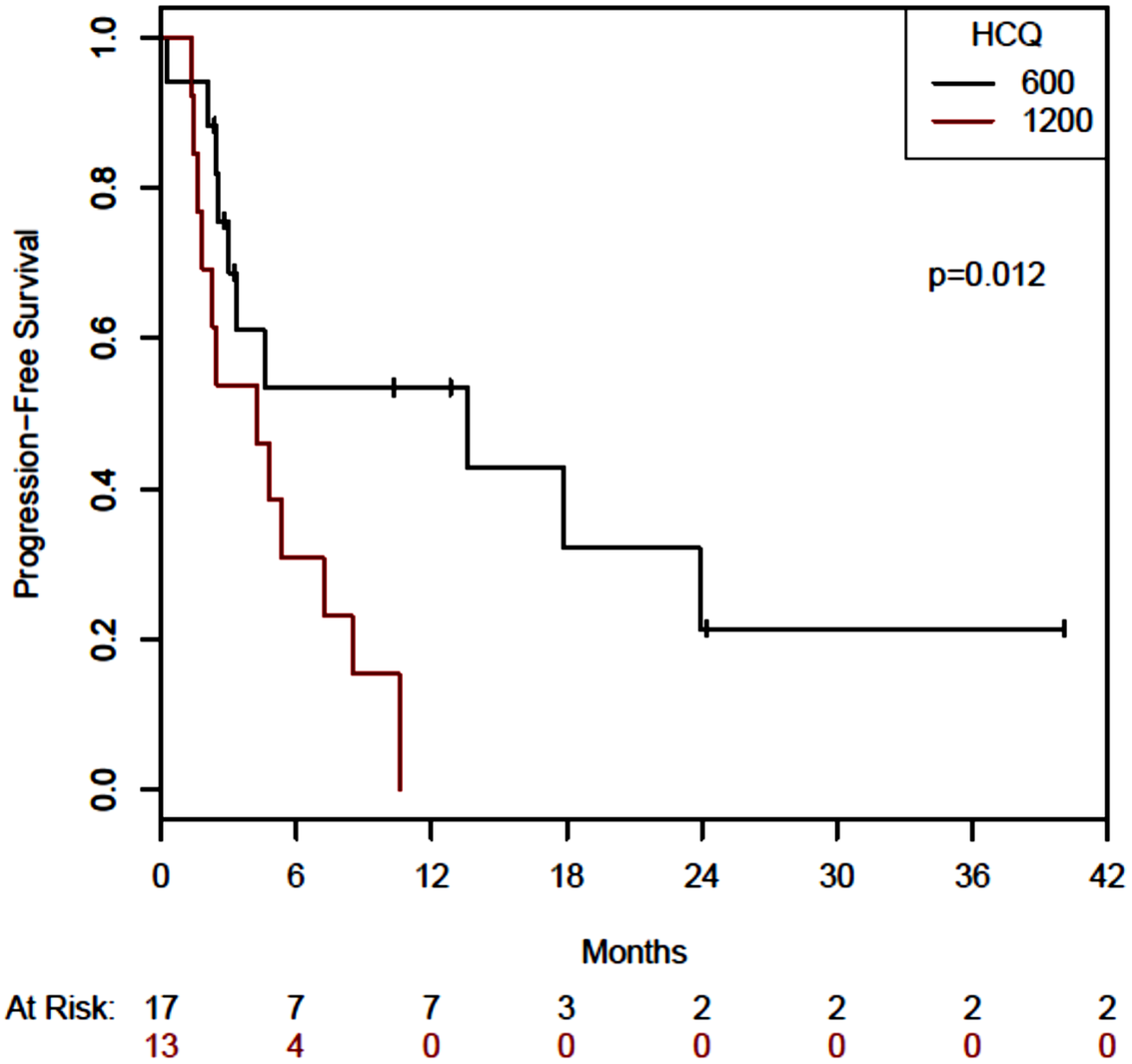
| **Gender** | |
|---|---|
| Male | 22 (71%) |
| Female | 9 (29%) |
| **Age** | |
| Median (Range) | 57.5 (45.2,68.8) |

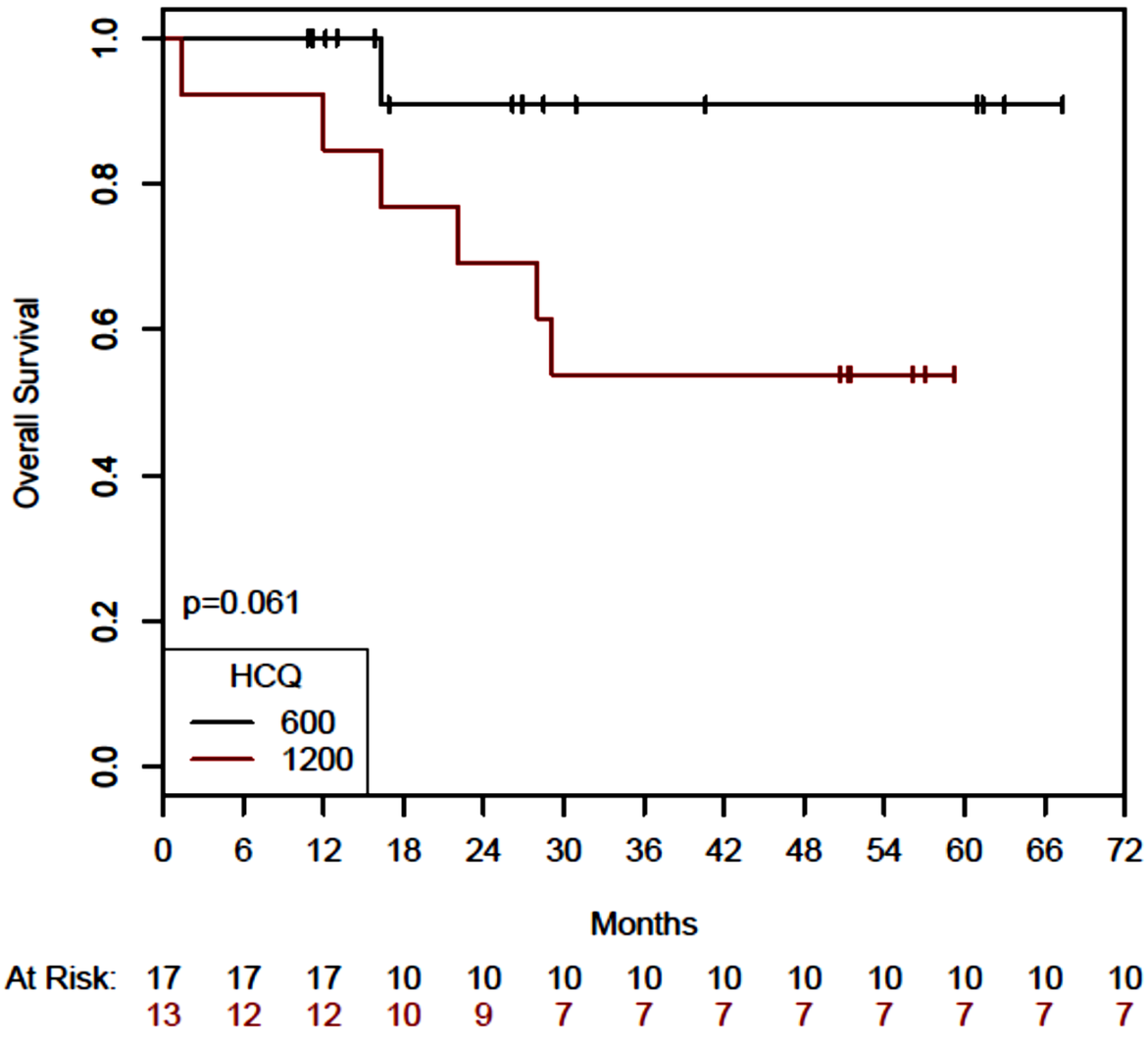
**Clinical Responses:** 3 subjects out of 29 evaluable (10%) had confirmed complete response (CR) as best response. 3 subjects had a partial response as best response.



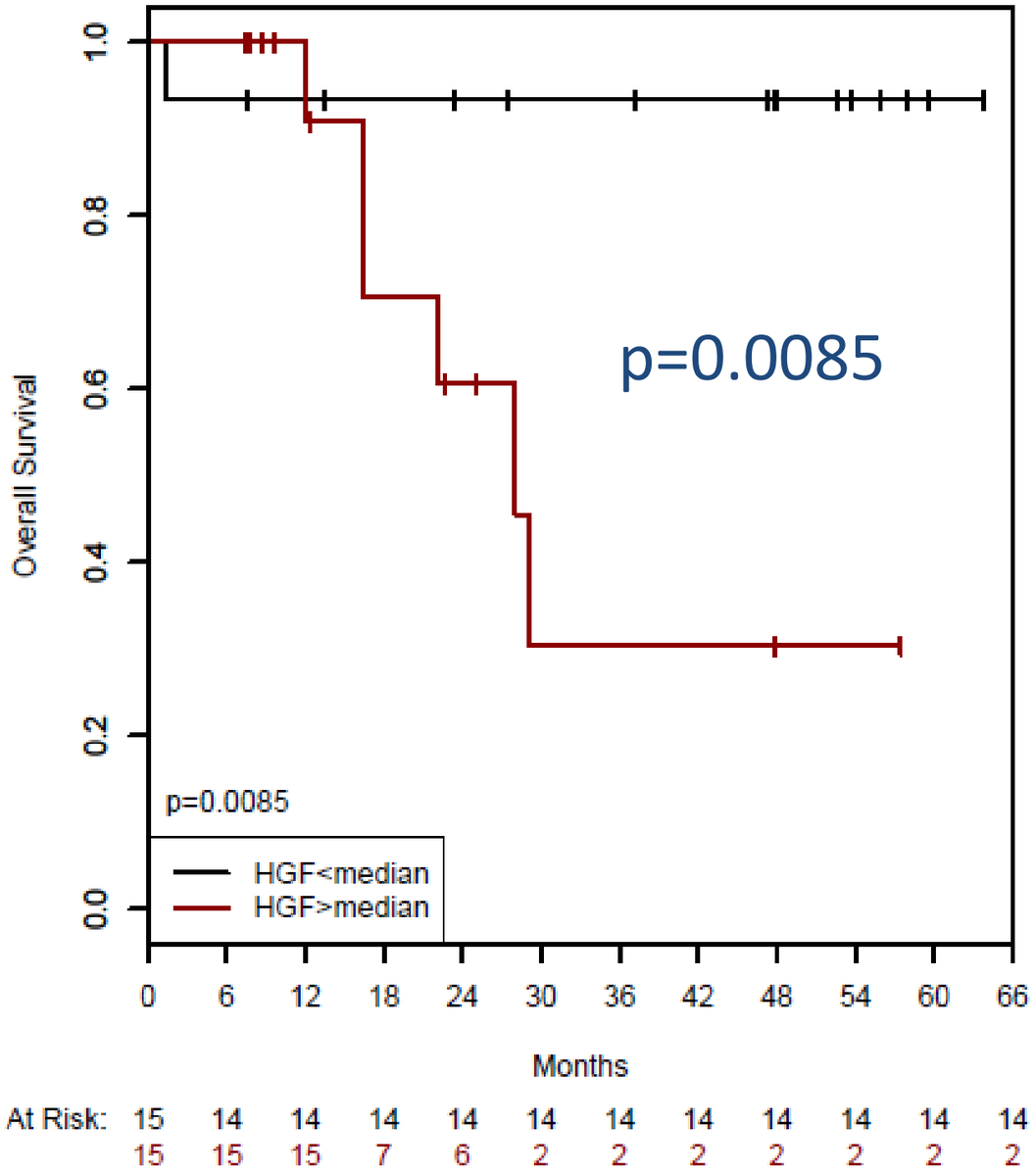

With first 2 weeks of HCQ, statistically increased BAFF, CXCL13, Eotaxin 1, 2, IL2Ra, MIG, sTNFRII, sBTLA in ThermoFisher Checkpoint 14-Plex ProcartaPlex™ & Immune Monitoring 65-Plex ProcartaPlex™ Human Panels.

# Improved PFS and Overall Survival – 600 mg HCQ



# Improved Survival with Serum Hepatocyte Growth Factor Less Than Median

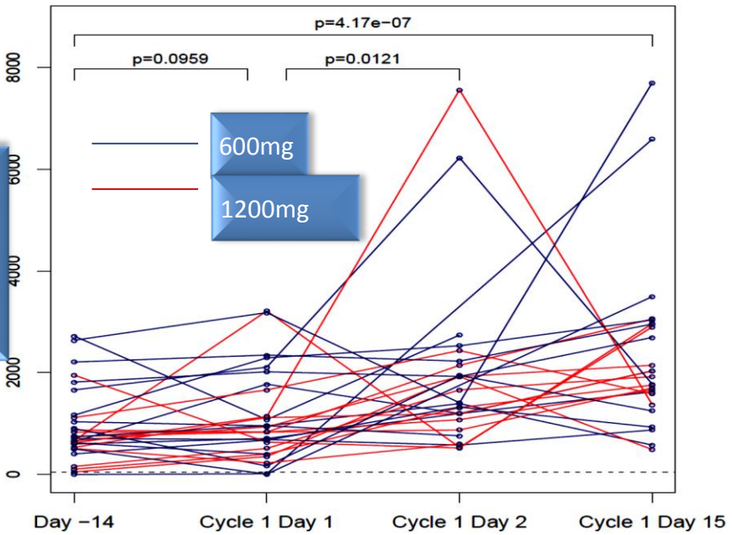



Tanimoto S, Fukumori T, El-Moula G, Shiirevnyamba A, Kinouchi S, Koizumi T, Nakanishi R, Yamamoto Y, Taue R, Yamaguchi K, Nakatsuji H, Kishimoto T, Izaki H, Oka N, Takahashi M, Kanayama HO. Prognostic significance of serum hepatocyte growth factor in clear cell renal cell carcinoma: comparison with serum vascular endothelial growth factor. J Med Invest. 2008 Feb;55(1-2):106-11.

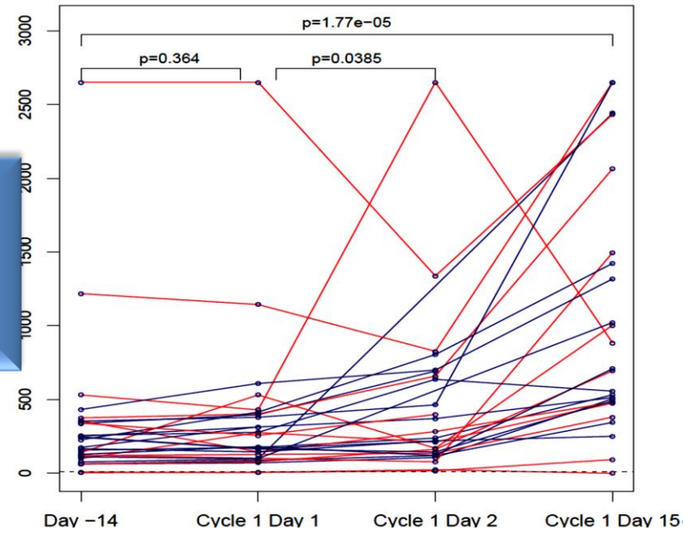
Survival of patients with high serum HGF (>1150 pg/ml) was significantly reduced compared to patients with low serum HGF concentrations (p=0.0044). In patients with nuclear grade 2 or high stage RCC, the higher serum HGF group exhibited significantly lower cause-specific survival (**p=0.0087 and p< 0.05, respectively**).

# Highly Significant Changes In Cytokines and Checkpoints

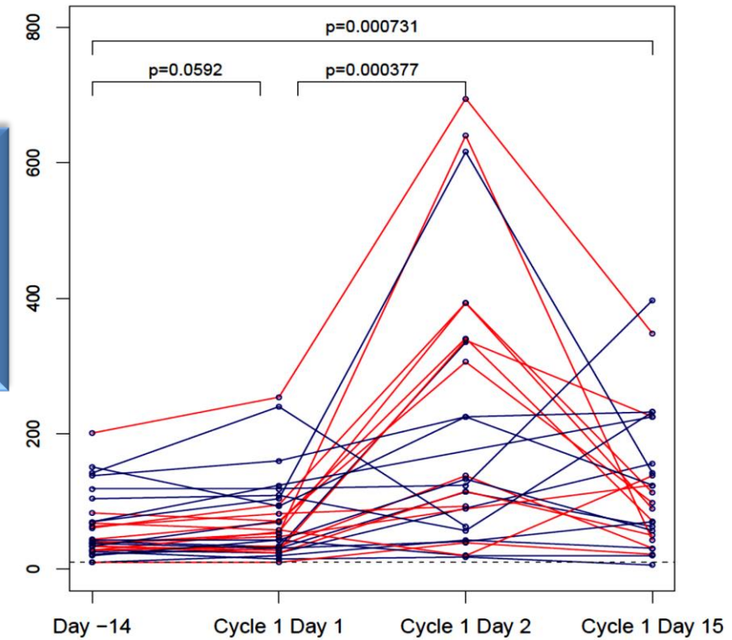
APRIL



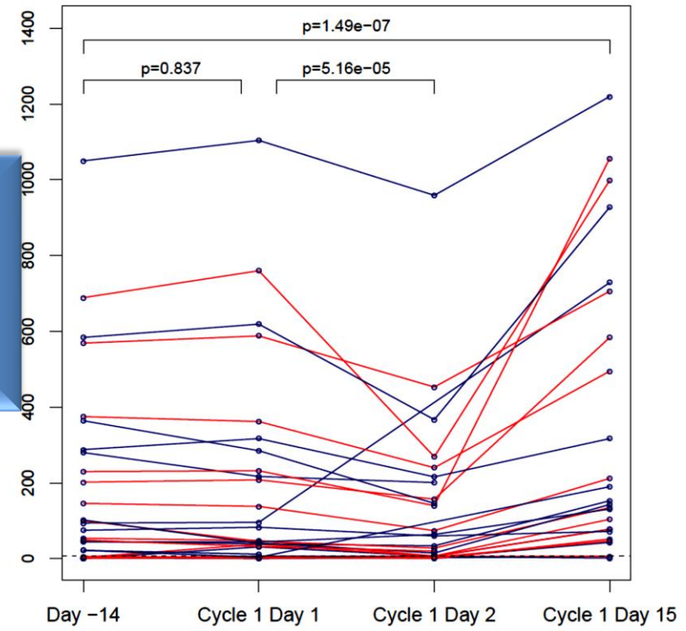

sCD30




sLAG-3




sCD40L

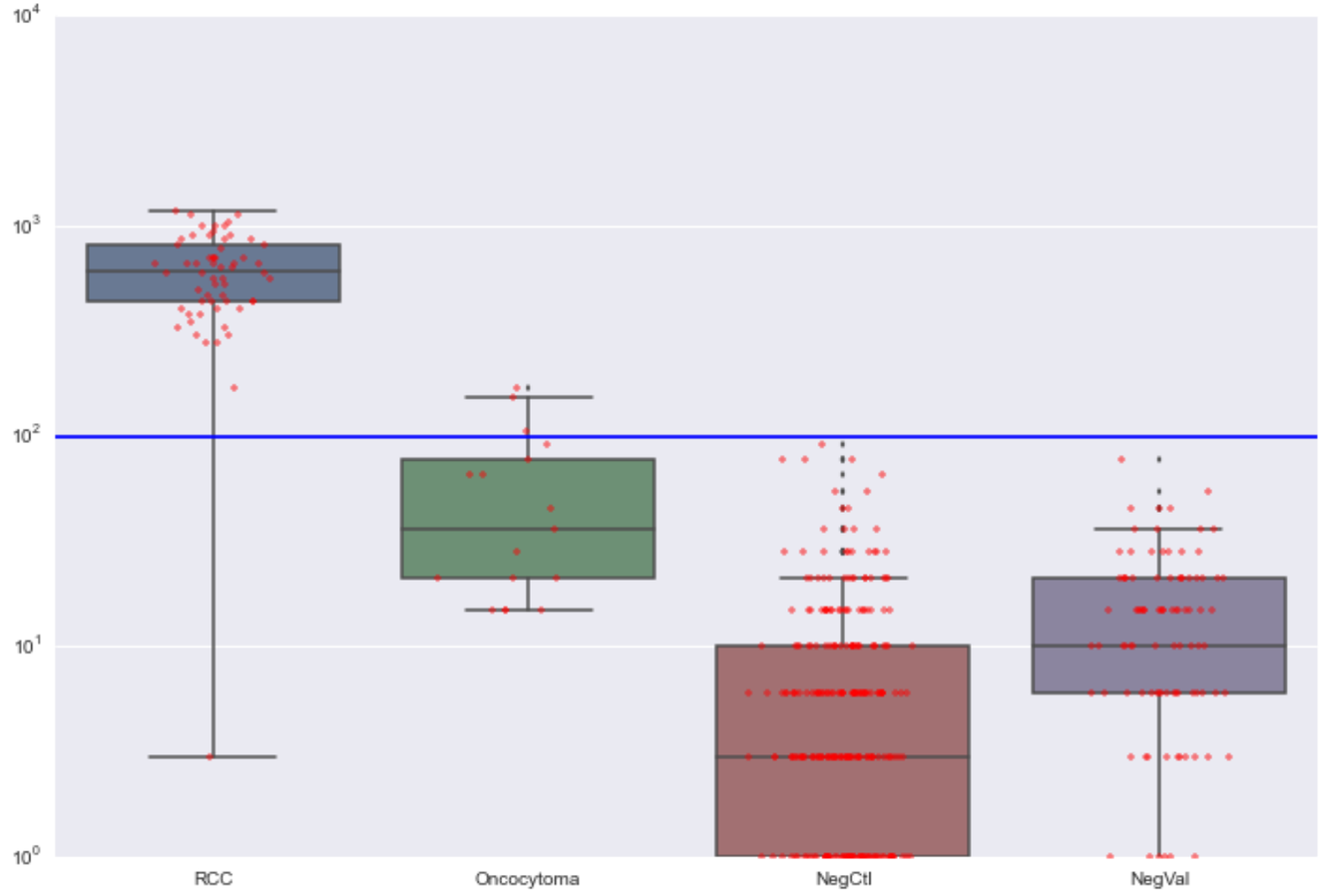


# Conclusions 2

| Accrual Period (Months) | Total n-2x | Total n/ Month | Total n-3x | Total n/ Month |
|---|---|---|---|---|
| **24** | 144 | 6 | 72 | 3 |
| **30** | 140 | 4.67 | 70 | 2.33 |
| **36** | 138 | 3.83 | 70 | 1.94 |
| **42** | 136 | 3.24 | 69 | 1.62 |

- The combination of high dose aldesleukin and daily oral hydroxychloroquine at a dose of 600 mg daily was well tolerated in patients with metastatic RCC
- No novel/increased toxicity of the combination was observed
- We have observed 3 confirmed CR and 3 PR in patients and ↑ PFS
- sLAG3, HGF>median are novel predictors of poor outcome
- Consider a randomized CWG Study - should be debated-high vs low dose IL-2; ? PEG-IL2/anti-PD1

# iRepertoire Shared CDR3 (DSLA) for Renal Cancer

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ARTICLE

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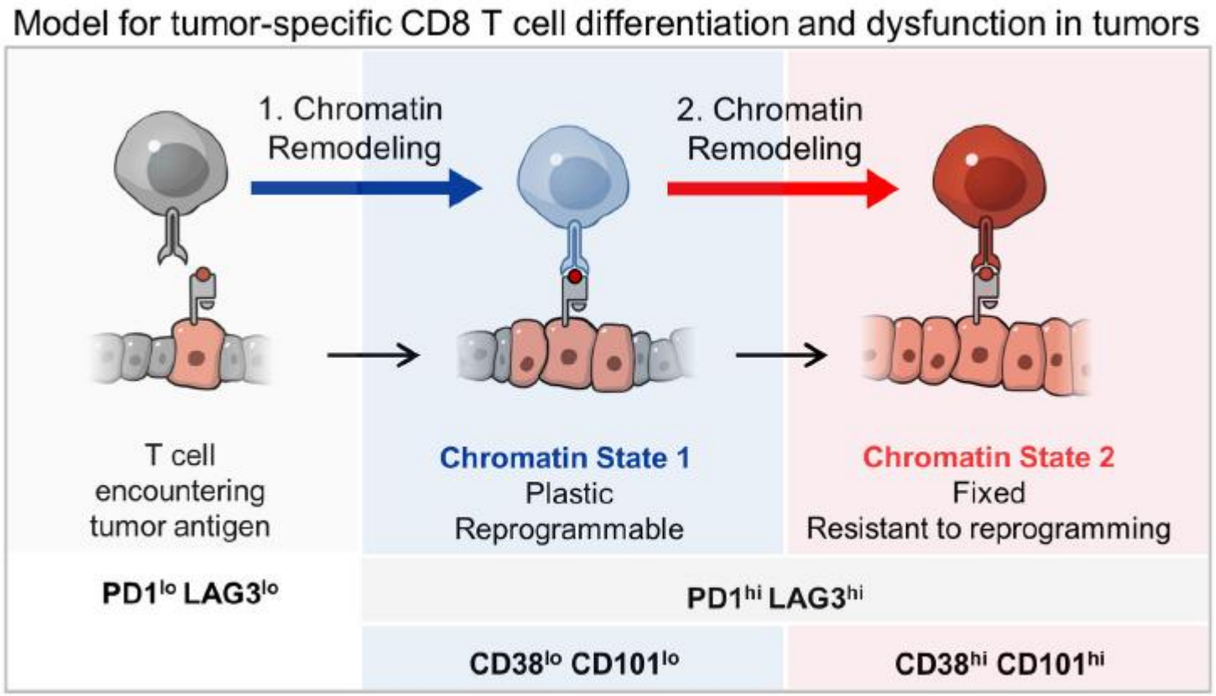
# Chromatin states define tumour-specific T cell dysfunction and reprogramming

Mary Philip[1], Lauren Fairchild[2,3], Liping Sun[4], Ellen L. Horste[1], Steven Camara[1], Mojdeh Shakiba[1,5], Andrew C. Scott[1,5], Agnes Viale[4], Peter Lauer[6], Taha Merghoub[5,7], Matthew D. Hellmann[5,8], Jedd D. Wolchok[5,7,9], Christina S. Leslie[2] & Andrea Schietinger[1,5]

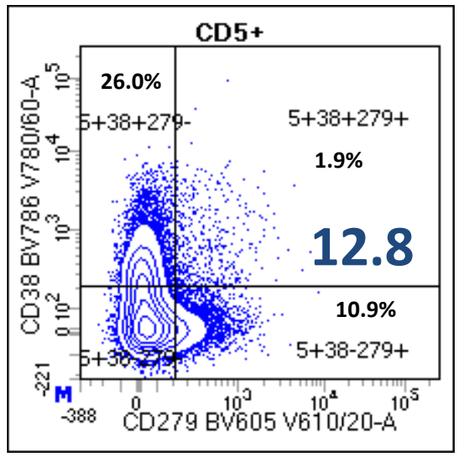



4 5 2 | N AT U R E | VO L 5 4 5 | 2 5 may 2 0 1 7

Immunogenomics Huntsville AL Oct 1-3

# Increased PD1+ CD5+ Cells in Peripheral Blood with IL-2 Treatment

CD38 ↑

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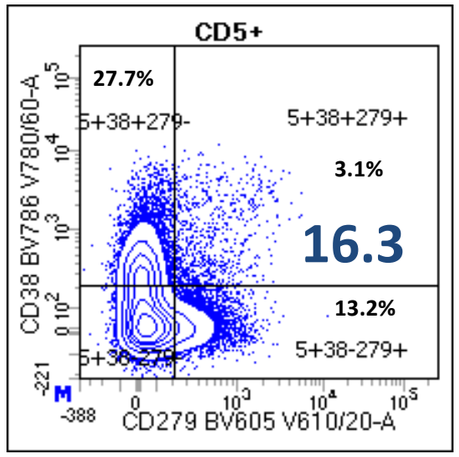
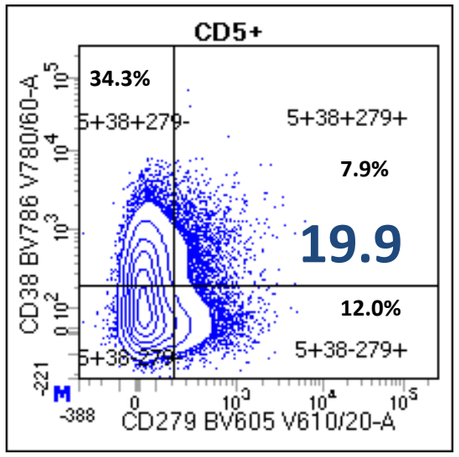
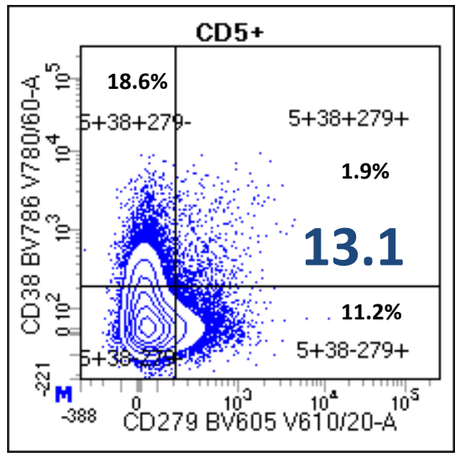



Post HCQ



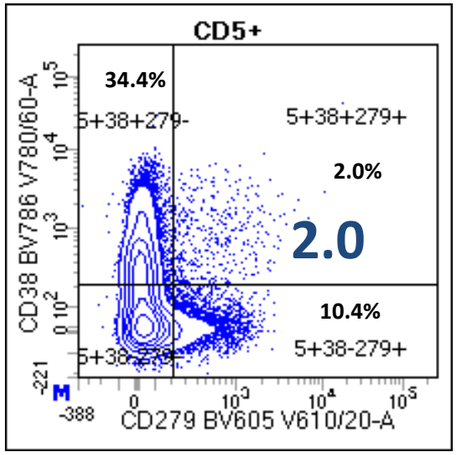

Post IL-2




Last

PD1 →

Subject 01-003 CR CD5+ T cells

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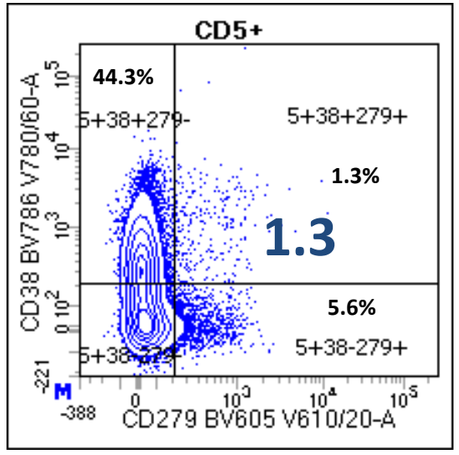
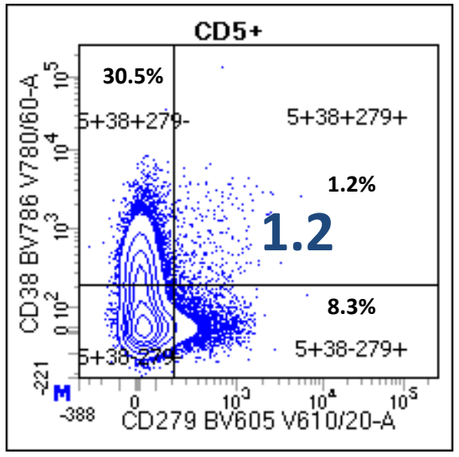



Post HCQ




Post IL-2

Subject 03-028 PR
CD5+ T cells

# Cytokine Working Group/Ryan Sullivan/IL-2 Proteomics Predictors

- DeepMALDI
  - Validated and standardized high throughput MS spectral acquisition
  - Samples:
    - Serum <10µl
    - Either from frozen aliquots or via serum cards
  - CLIA ready
- dxCortex
  - Machine learning algorithms based on deep learning techniques
    - Avoid over-fitting
    - Development performance estimates ≈ validation estimation (in 47 projects)
      - Evaluation only on test part of development data
    - Enables design to purpose

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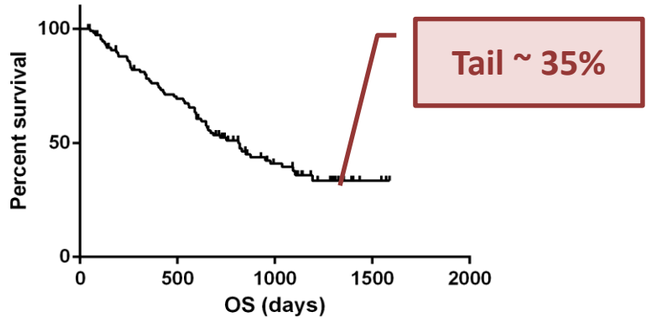
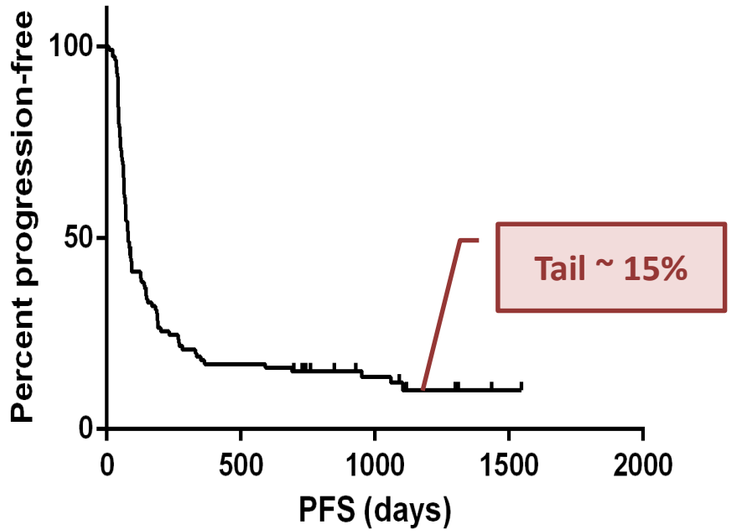
# Identifying IL-2 Responders in Melanoma Patients

## Samples from R Sullivan (MGH)

- N=114; Median PFS = 79 days, Median OS= 813 days
- No benefit rate: 60PDs ~50%
- Likely tx with checkpoints
  - After IL2 failure, see OS

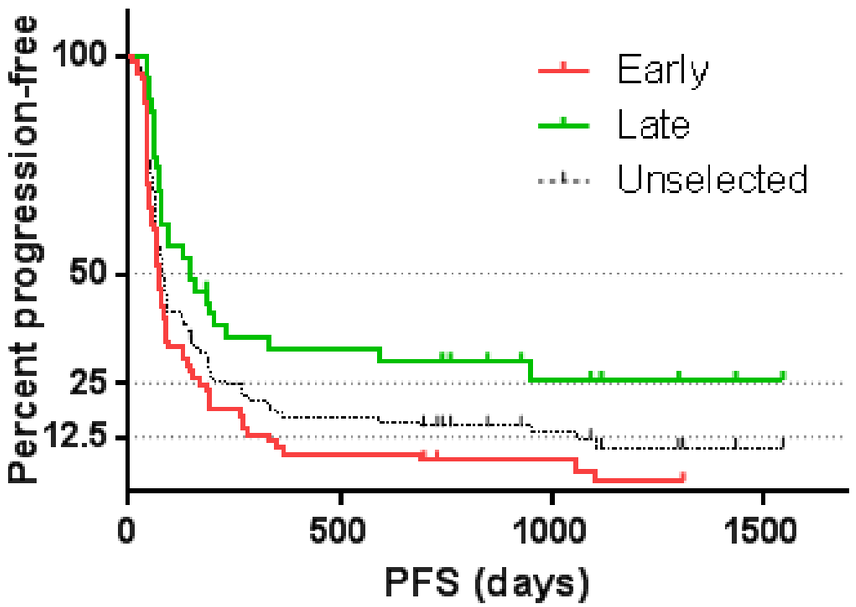



| | n (%) |
|---|---|
| CR | 8 (7) |
| PR (overall) | 13 (11) |
| PR (PFS <1 yr) | 8 (7) |
| PR (PFS >1000days) | 1 (1) |
| PR(PFS, no event) | 4 (4) |
| Minimal Response | 6 (5) |
| SD | 26 (23) |
| PD | 60 (53) |
| NE/NA | 1 (1) |

# Distinguishing Before Therapy Responders and Progressors

## Response and PFS



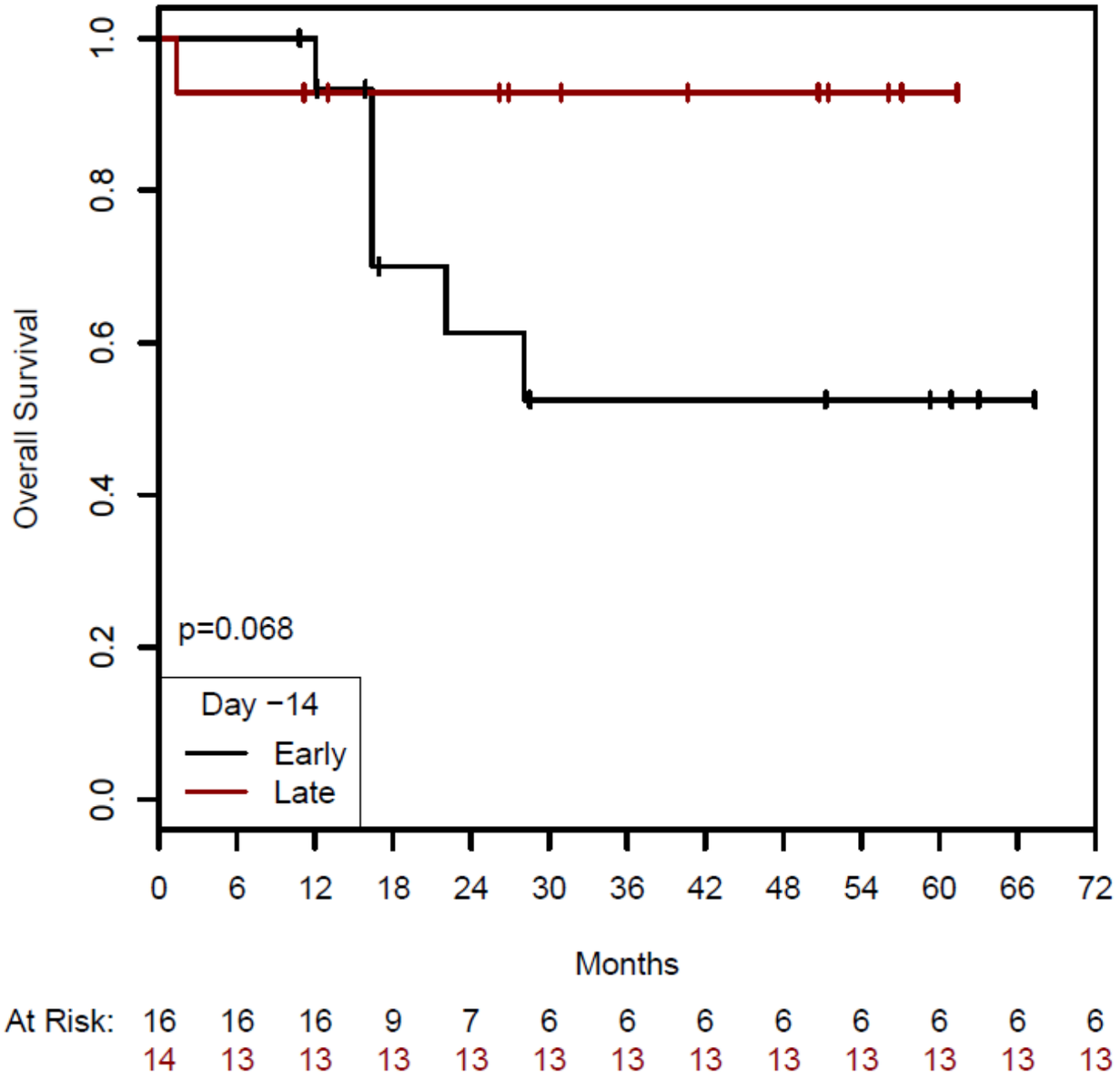

| | PFS | | |
|---|---|---|---|
| | log-rank p | CPH p | HR (95% CI) |
| Early vs Late | 0.001 | 0.001 | 2.12 (1.36-3.30) |

| | Early (N=75) | Late (N=39) |
|---|---|---|
| PD | 44 (59%) | 16 (41%) |
| SD | 17 (23%) | 9 (23%) |
| Minimal Response | 3 (4%) | 3 (8%) |
| PR (all) | 10 (13%) | 3 (8%) |
| PR (PFS < 1yr) | 7 (9%) | 1 (3%) |
| PR (PFS > 1000 days) | 1 (1%) | 0 (0%) |
| PR (PFS, no events) | 2 (3%) | 2 (5%) |
| CR | 0 (0%) | 8 (21%) |
| NE/NA | 1 (1%) | 0 (0%) |

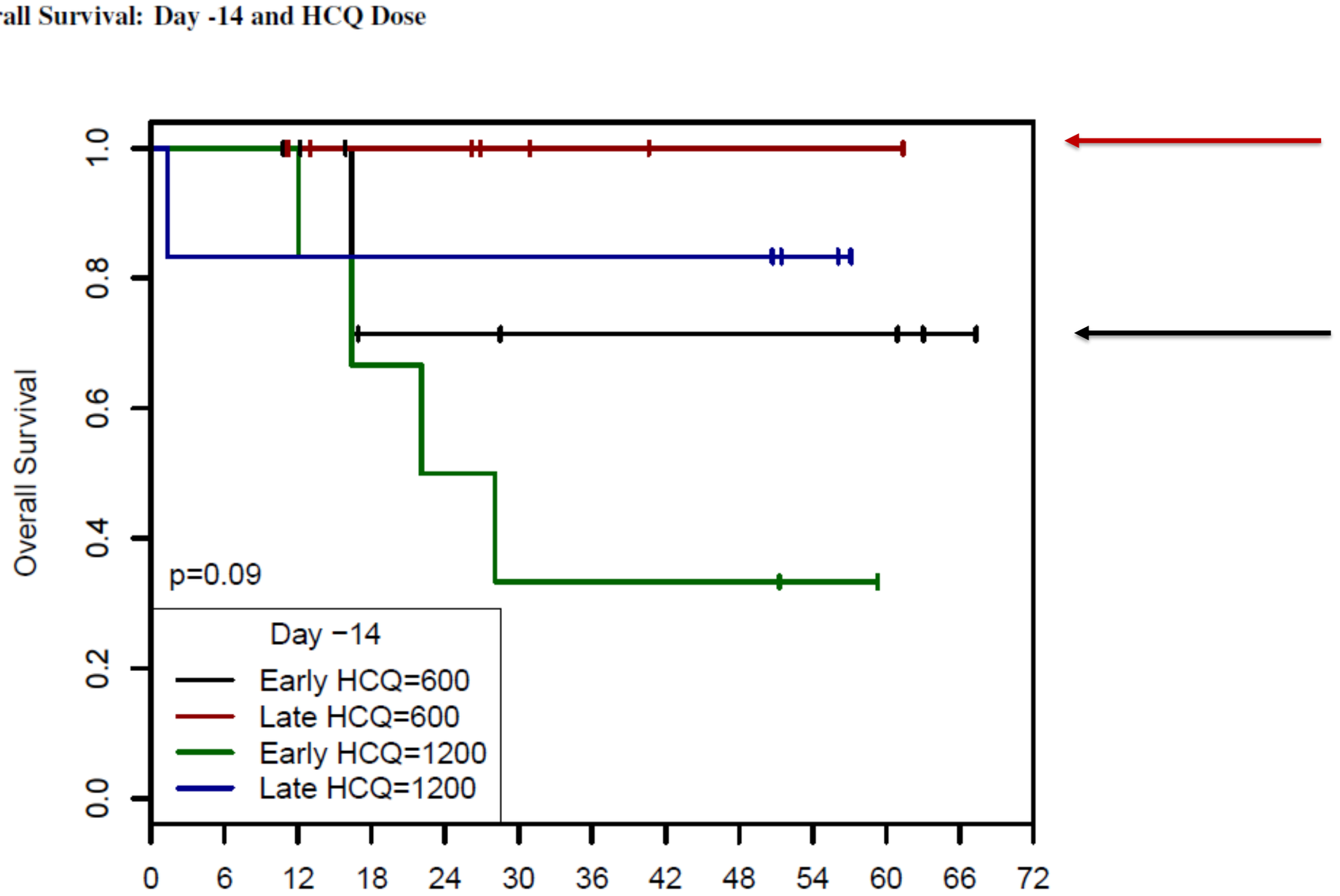
| | Early (N=75) | Late (N=39) |
|---|---|---|
| Response | 13 (17%) | 14 (36%) |
| No response | 62 (83%) | 25 (64%) |

- ORR is significant: p=.037
- DCR trends: p=.075
- CR rate in Lates (21%)

# Predicting RCC Survival with Proteomics



# Predicting RCC +/- HCQ Survival with Proteomics



# Conclusions 3

- Multiplexed robust PCR for TCR and BCR can potentially identify a signal in renal cancer and is being tested in our current cohort of patients (?SITC 2018) – Jian Han presenting tomorrow
- PD-1 is a marker of antigen activation, subsetted by two variable phenotypes – open and closed chromatin; examining these in the blood, draining lymph nodes and tumor will be of importance
- Novel proteomic application to predict IL-2 responsiveness could be useful in identifying those patients most likely to respond and potentially identify novel DAMPs in the circulation that could correlate with response