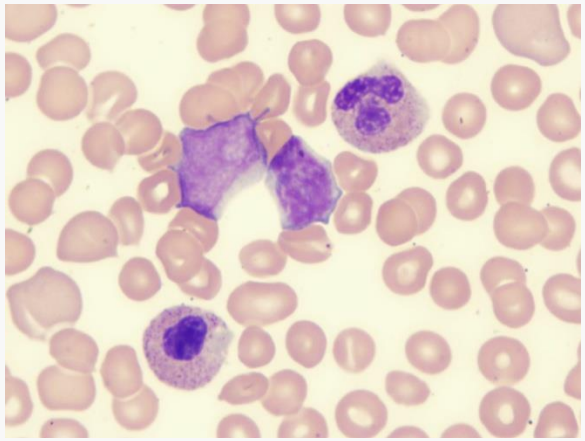
Anti-MDS Immunity: a potential player in the response to hypomethylating agents

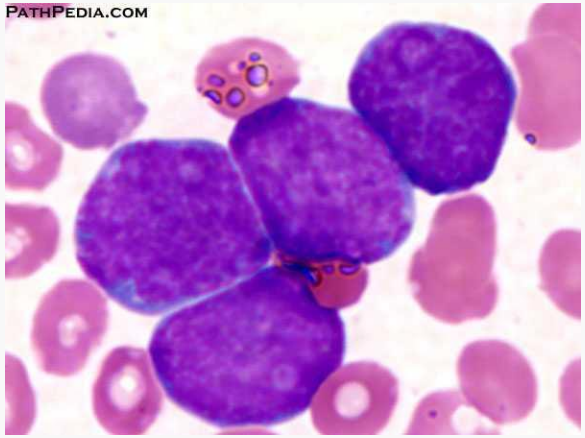
Elizabeth A. Griffiths, MD
Associate Professor
Roswell Park Cancer Institute
State University of New York at Buffalo
Medicine, Immunology & Pharmacology

Recognized Prognostic Factors in MDS and AML

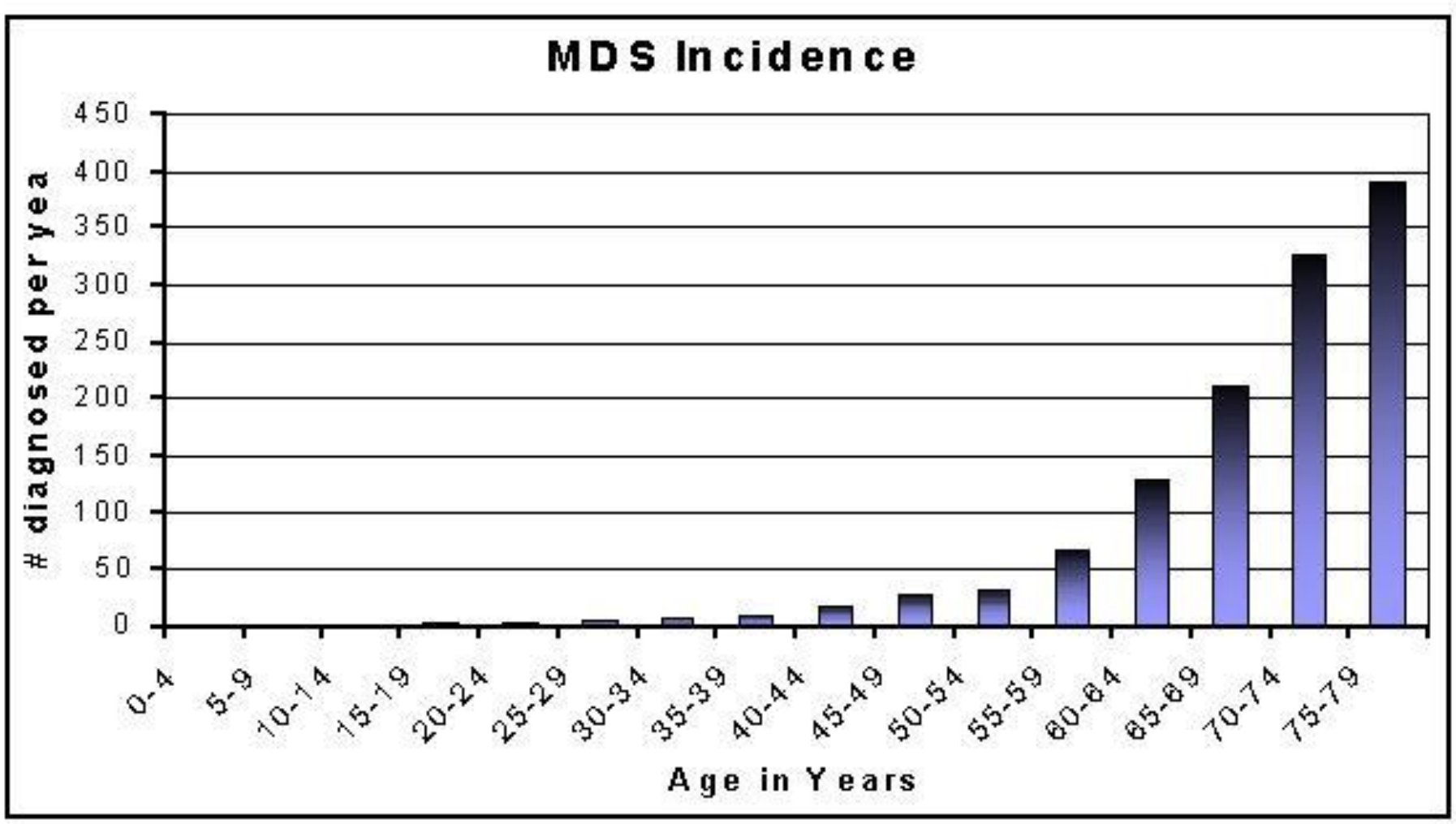
- MDS
  - Age
  - PS
  - Cytopenias (Hg<10, Plt<100, ANC<1K)
  - Bone marrow blast percentage (>20% = AML)
  - Cytogenetics(-5,-7, complex, poor risk)
  - Median survival 0.4-5.7yrs



- AML
  - Age
  - PS
  - Cytogenetics (-5,-7, complex, poor risk)
  - Antecedent hx of MDS
  - Molecular Markers (NPM1, FLT3, CEBPa)
  - Median Survival 1.5-2yrs

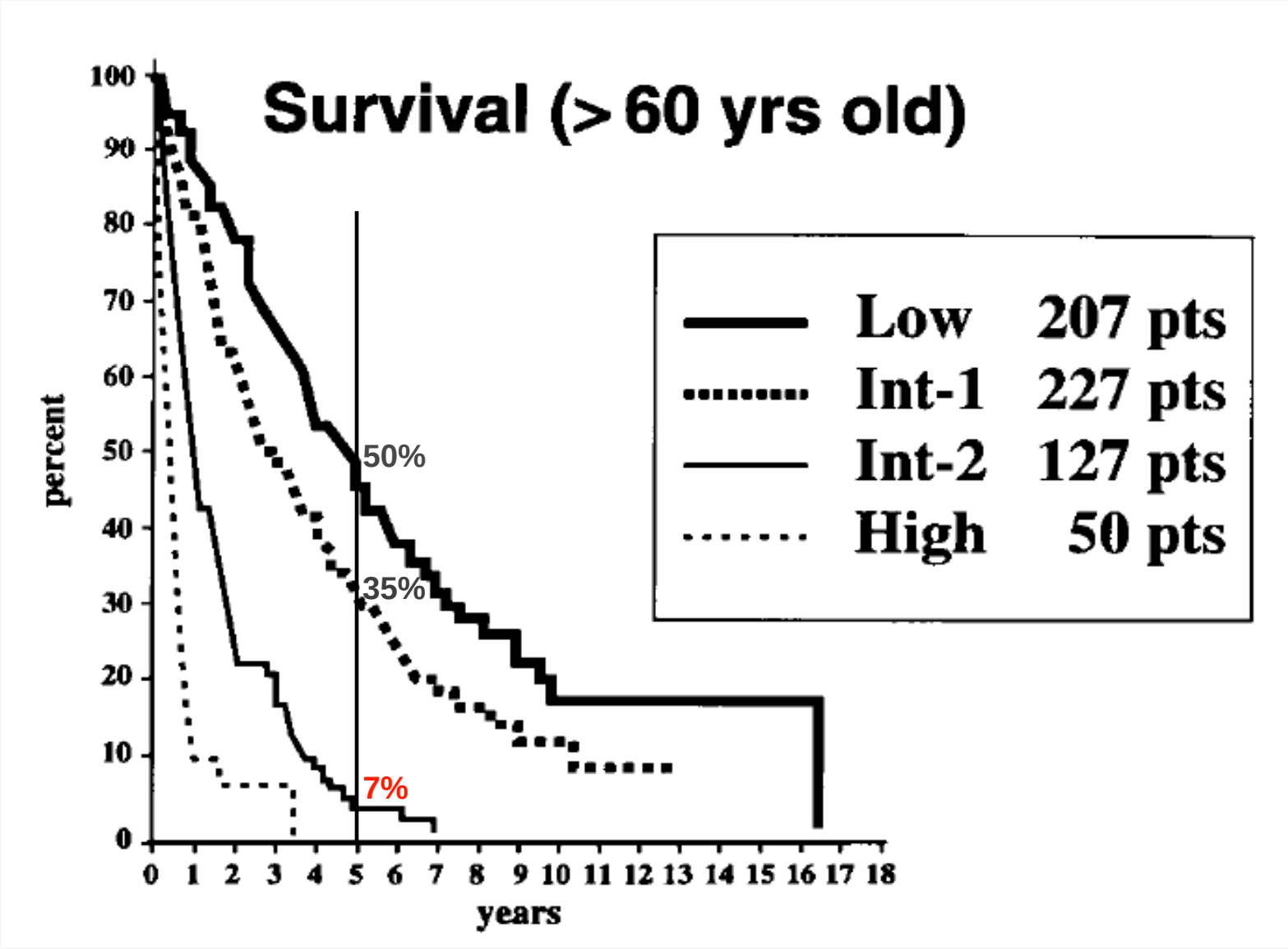


Incidence of MDS as a Function of Age

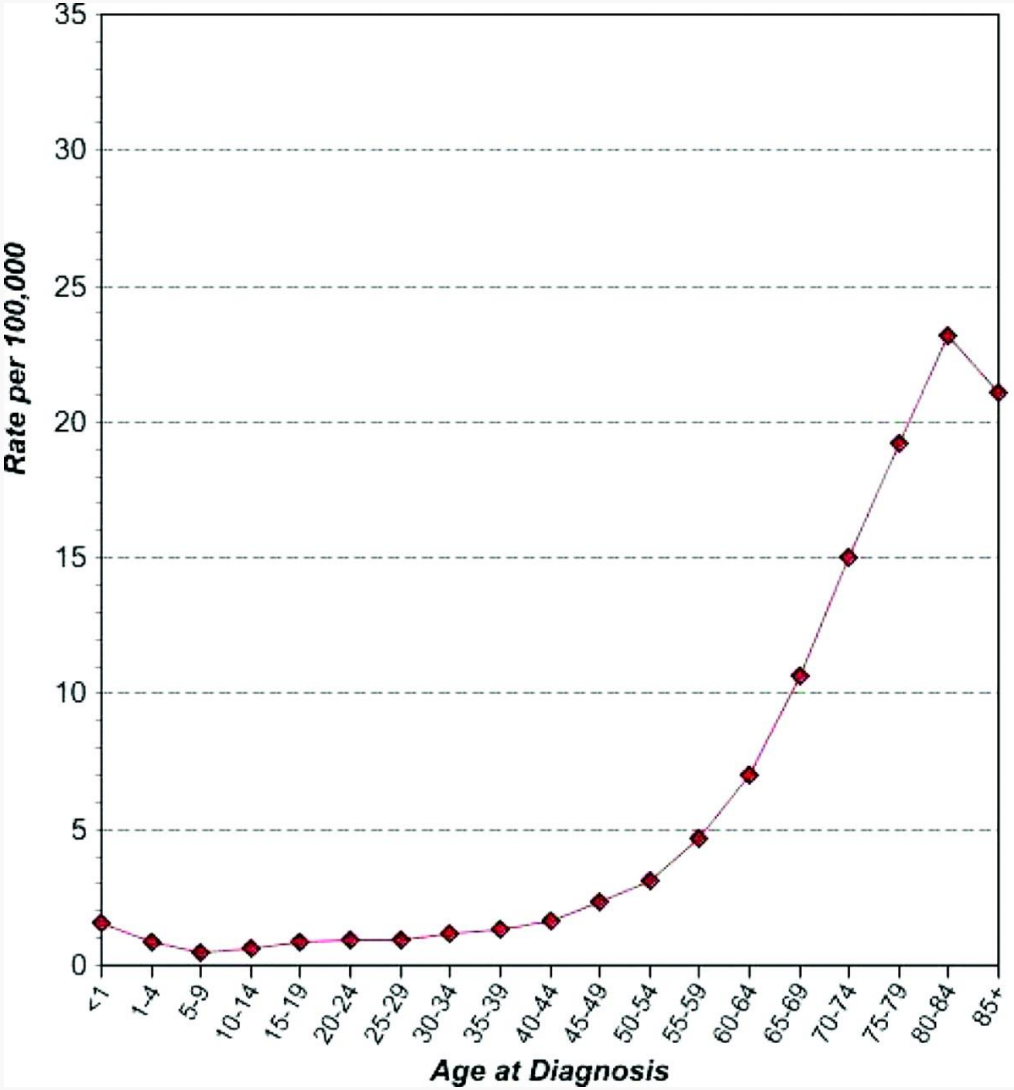


Phekoo et al. Haematologica. 2006;91:1400-4.

Outcome of MDS >60 years old

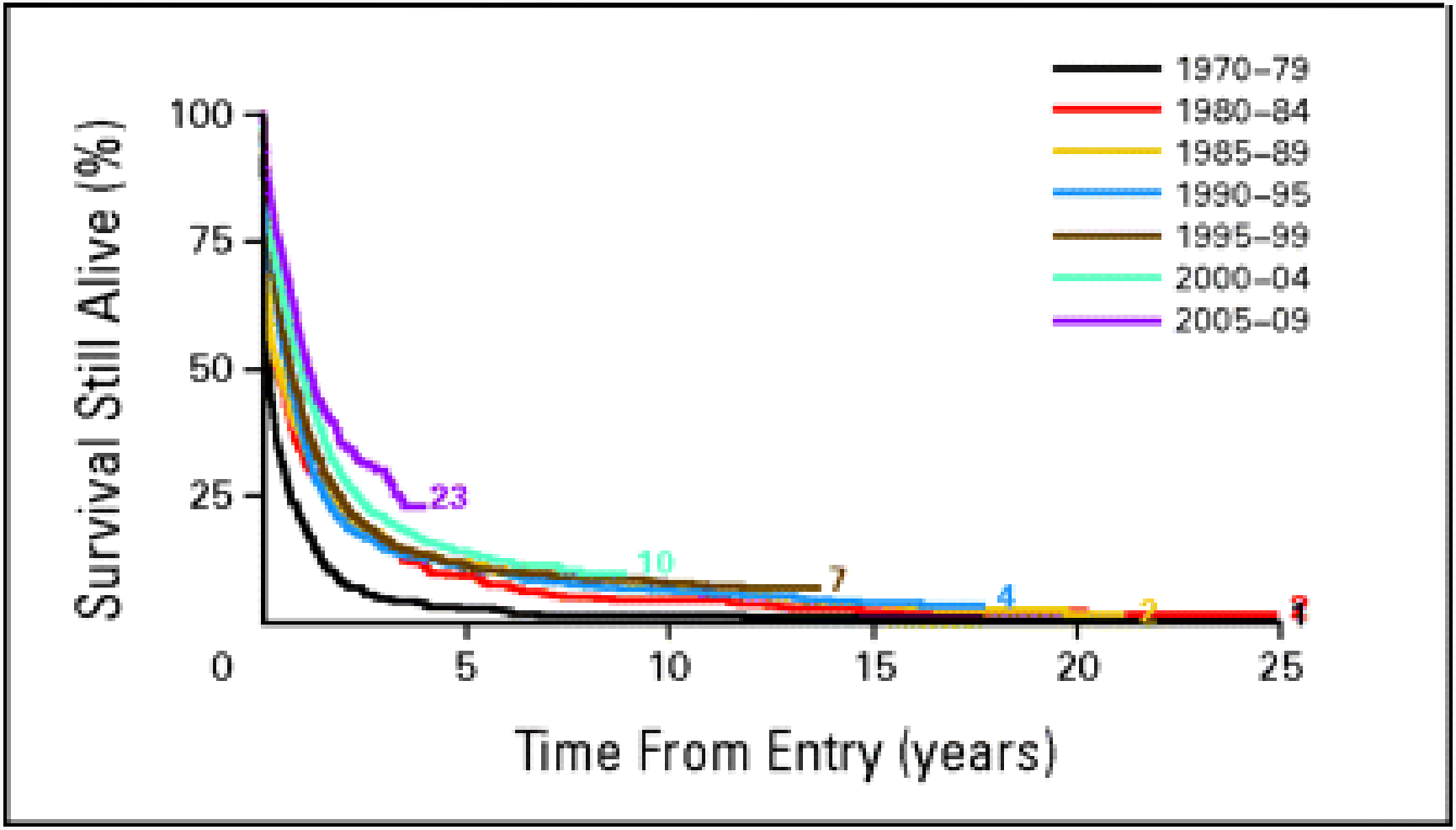


Incidence of AML as a Function of Age



Klepin H D et al. The Oncologist 2009;14:222-232.

Outcome of AML >60 years old

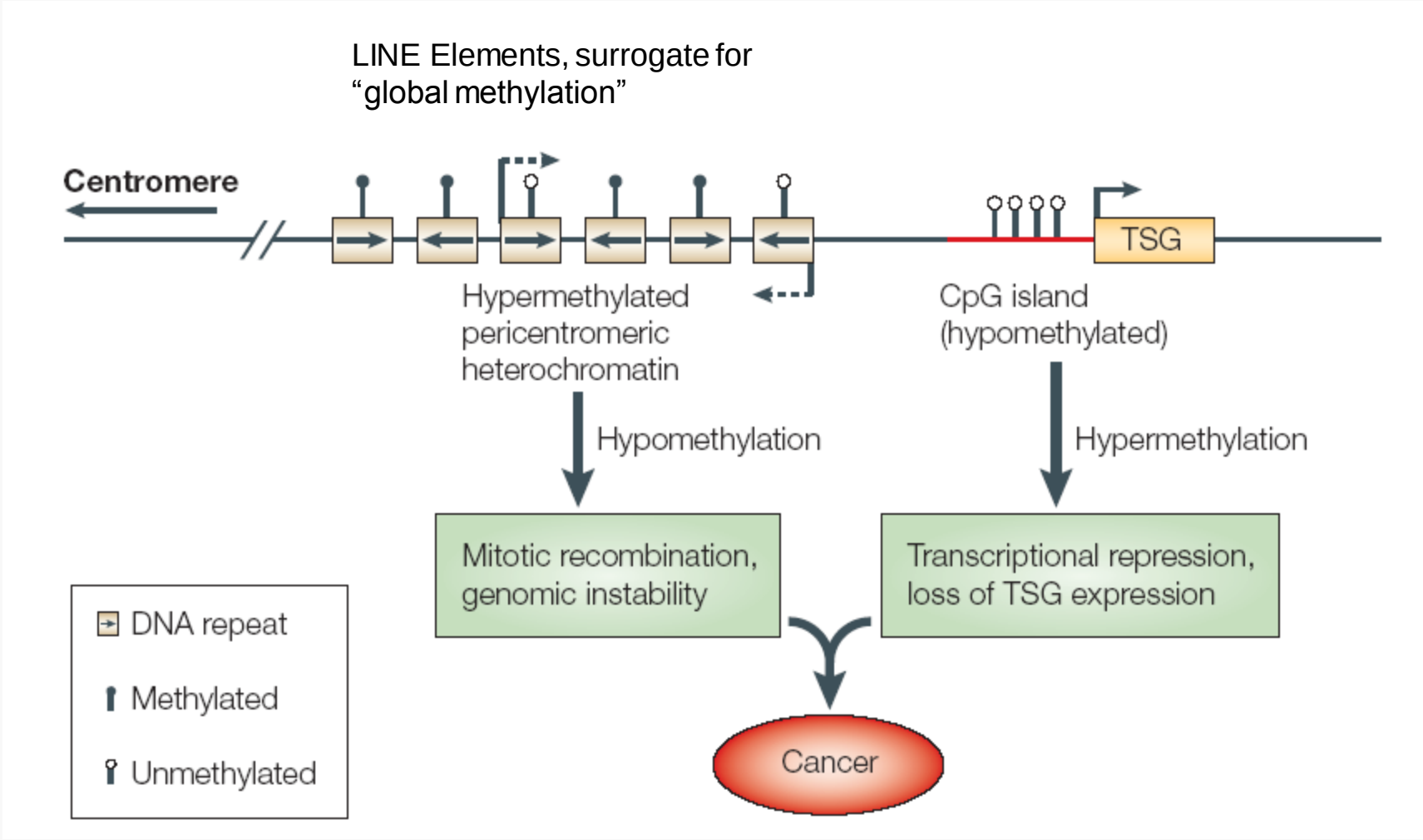


Burnett et al. JCO 2011;29:487-94.

Azacitidine (Aza) and Decitabine (Dac)

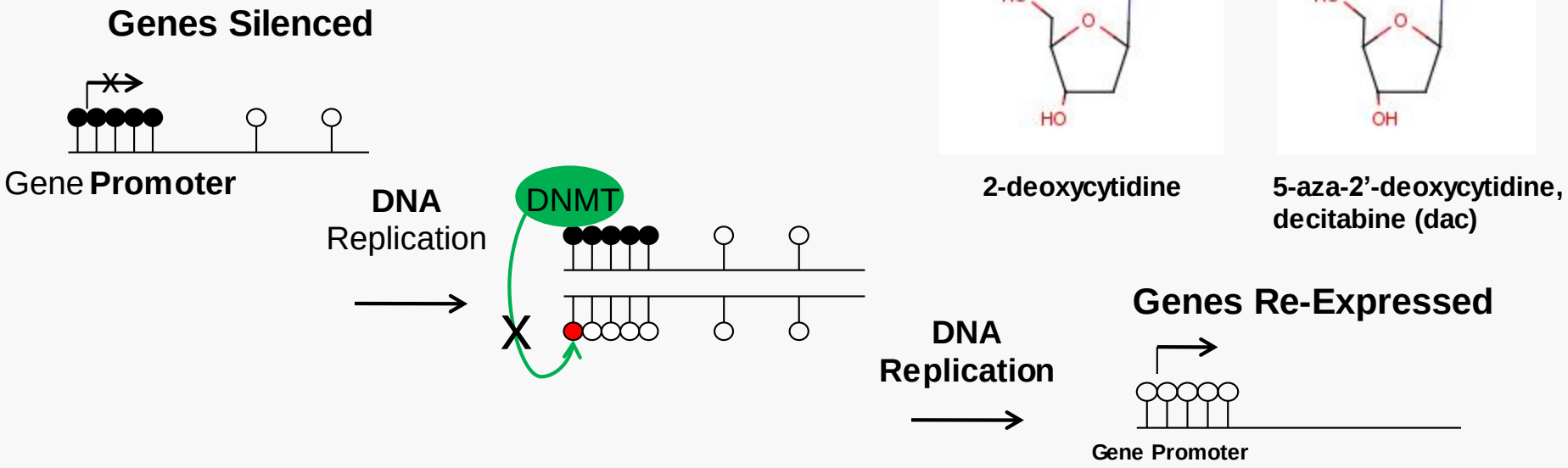
- FDA approved for MDS, off label for AML
- Prolong SURVIVAL, but take months to work
- Mechanisms remain controversial and include:
  - Re-expression of epigenetically silenced tumor suppressor genes (*p15INK4B, DAPK, p73*)
  - Direct cell kill (DNA double strand breaks)
  - Immune modulation and/or induction of autologous responses to induced antigens

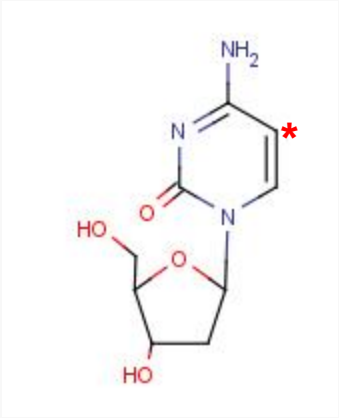
DNA Methylation in Normal and Cancer Cells



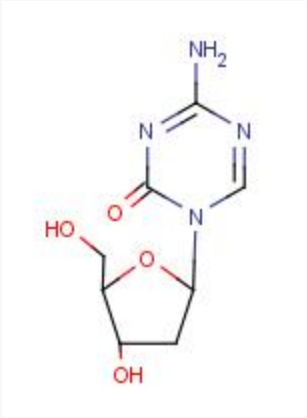
Robertson KD, Nat Rev Genet 2005;6:597-610.

Hypomethylating Drugs (HMAs) Reverse Methylation and Re-Express Genes





2-deoxycytidine



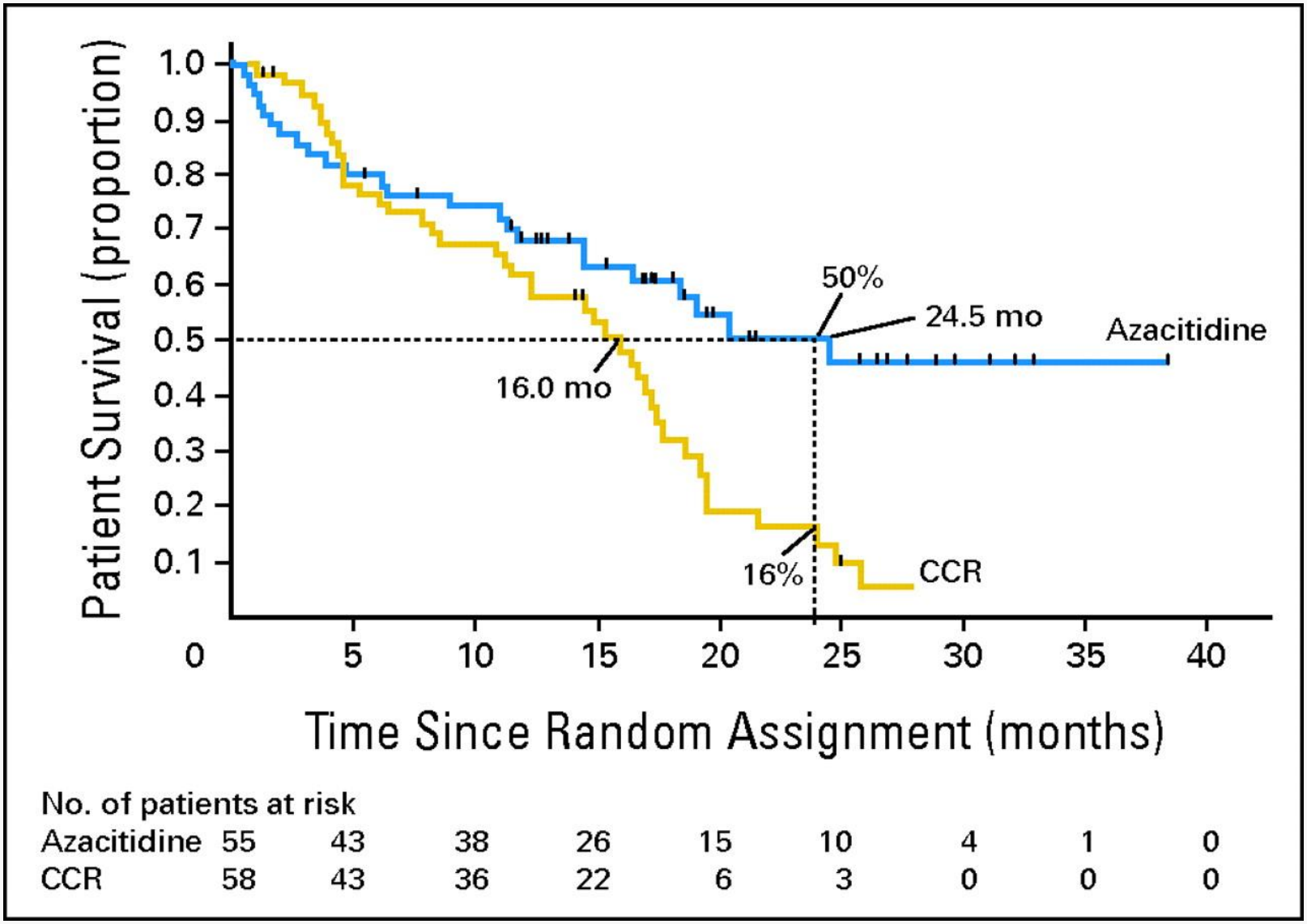
5-aza-2'-deoxycytidine, decitabine (dac)

HMAs incorporate into DNA and act as a suicide substrate for cellular enzymes that maintain methylation signatures

Pre-HMA Options

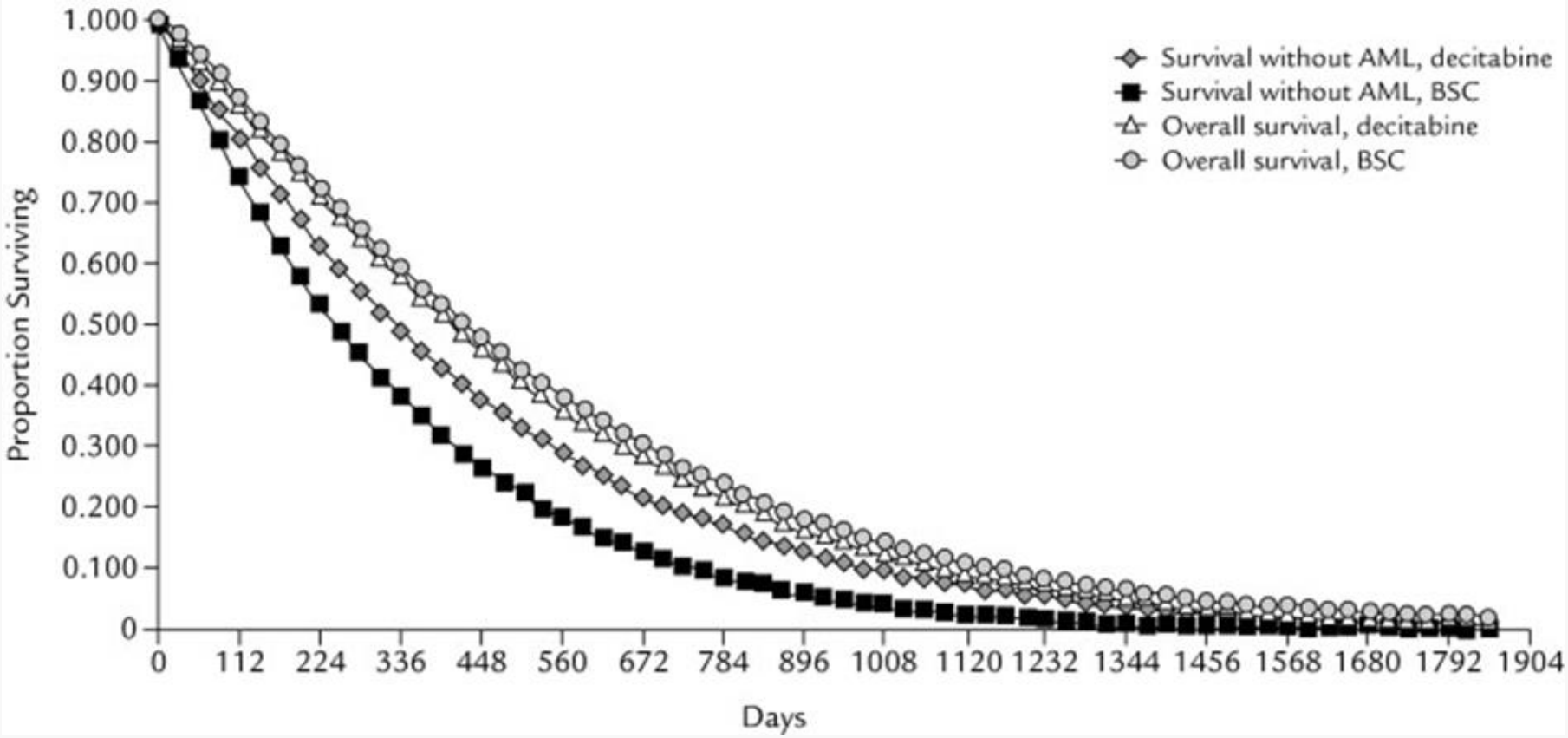
- **Induction chemotherapy with "7+3" chemotherapy**
  - **Highly toxic**
  - **One month hospital stay**
  - **Profound cytopenias**
  - **High infection rates**
  - **Induction failure is high (~50% CR)**
- **Low dose cytarabine**
  - **10-20% CR rate**
  - **Outpatient**
  - **Short duration of response**
- **Supportive care**
  - **Hydrea to manage hyperleukocytosis**
  - **Transfusion support**
  - **Antibiotics**

OS for AML Aza vs CC



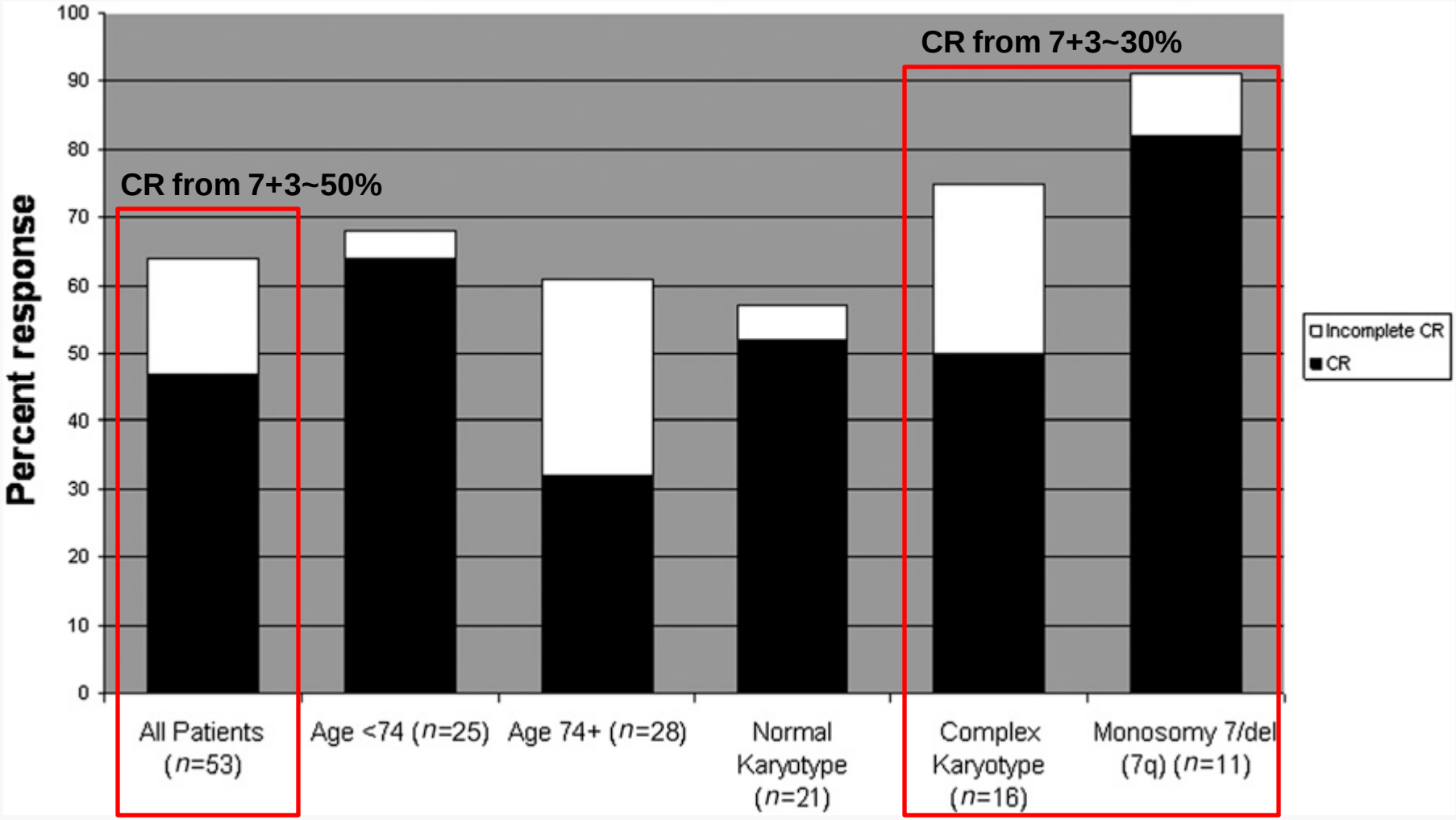
Fenaux P et al. JCO 2010;28:562-569.

Survival for MDS Pts Treated w/Dac



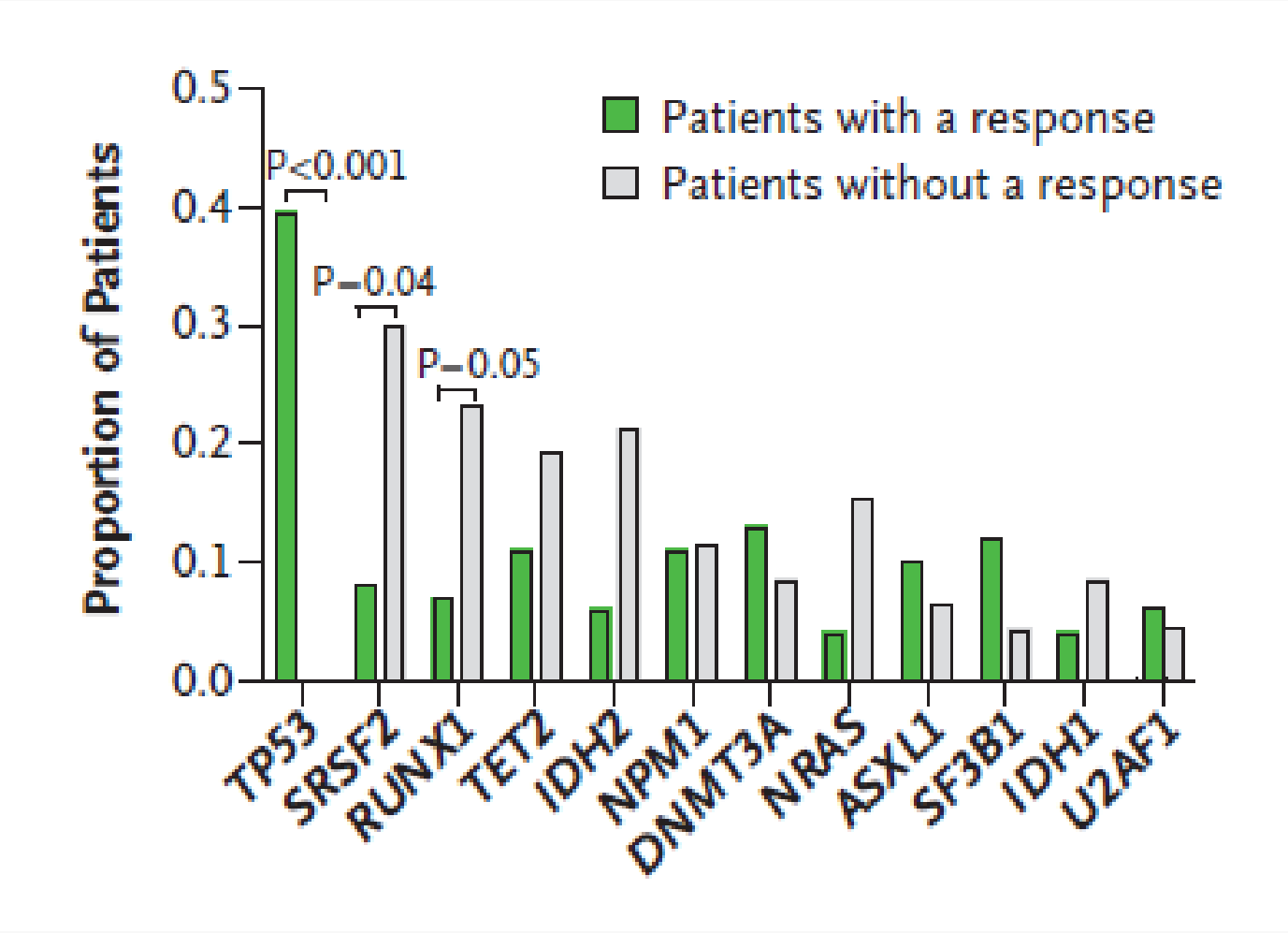
Pan et al. Clinical Therapuetics 2010; 32:2444-56.

Dac in AML Unfit for Induction



Blum et al. PNAS 2010; 107:7473-78.

p53^{mut} and HMA response



N=99 patients Response to Dac by mutation: P53^{mut} 21 of 21 [100%] vs. Others: 32 of 78

Welch JS et al. NEJM 2016;375:21

Hypothesis

- Anti-MDS- directed CD4 and CD8 T-cells contribute to the clinical response to HMAs in patients with myeloid malignancy

Gap in the Field

- Patients with MDS have evidence of auto-immunity which correlates with lower risk disease
- ~50% of patients respond to HMA therapy
  - Responses comprised of 15% CR; 35-40% HI, take MONTHS
  - no correlation between gene specific/global hypomethylation and response
  - No correlation between cytotoxicity and response
- **Mechanism** controversial; cell cycling **required**

HMAs: Azacitidine (Aza) and Decitabine (Dac)

- FDA approved for MDS, off label for AML
- Prolong SURVIVAL, but take months to work
- Observations demonstrate:
  - Re-expression of epigenetically silenced tumor suppressor genes (*p15INK4B, DAPK, p73*)
  - Direct cell kill (DNA double strand breaks)
  - Maybe: Immune modulation and/or induction of autologous responses to induced antigens

Cancer Germline Antigens

- ~150 genes, X-linked and autosomal
- Expressed ONLY in the embryonic ovary and adult testis, hypermethylated and silenced in normal adult tissues
- Aberrant expression in some cancers, due to hypomethylation of the gene promoters
- Cell-mediated and humoral immunity *de novo* in expressing cancers, associated with slower disease progression
- Vaccines phase I-III clinical trials in cancers with endogenous gene expression: *eg* MAGE-A3 (Lung), NY-ESO-1 (Ovary)

Why No CG Specific Immunotherapy for Myeloid Cancer?

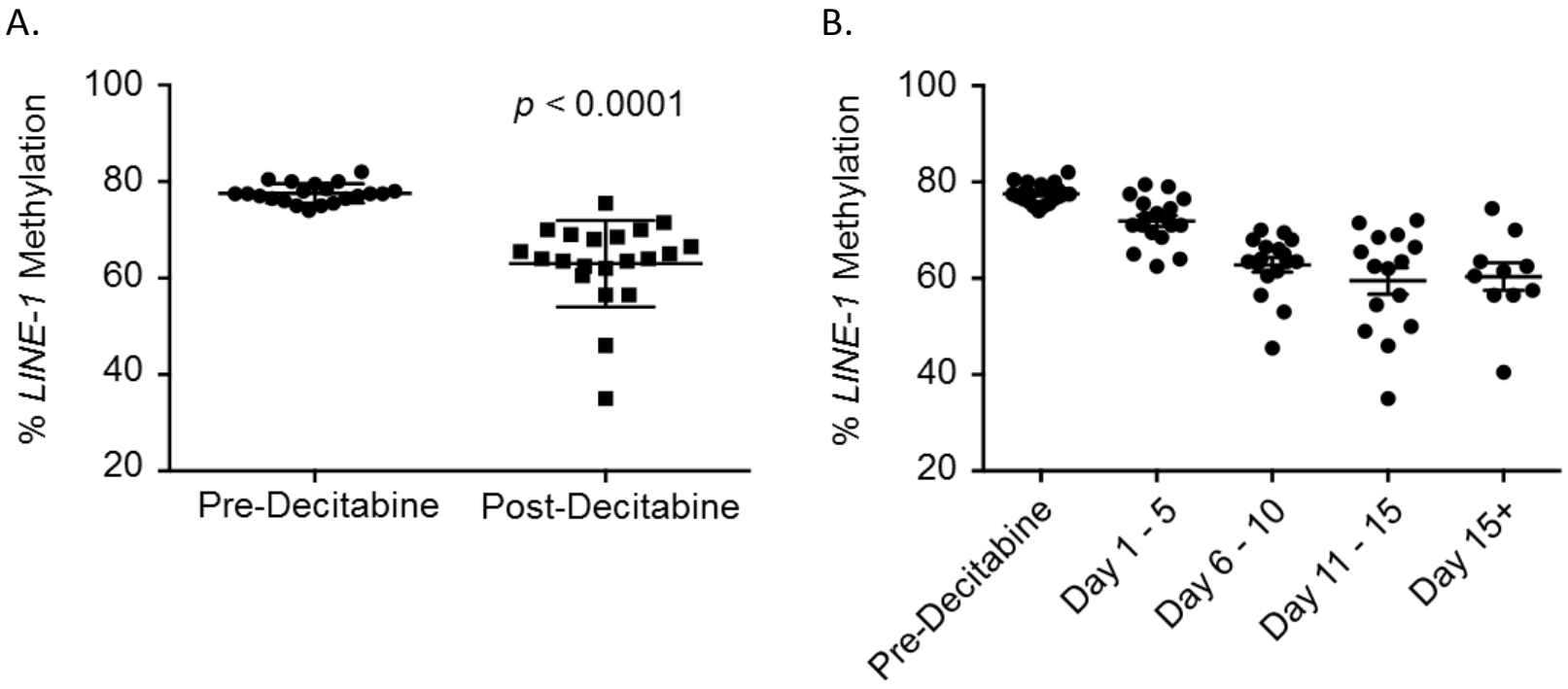
- Not usually expressed

- Dense hypermethylation of CG antigens promoters results in gene silencing in most heme malignancies

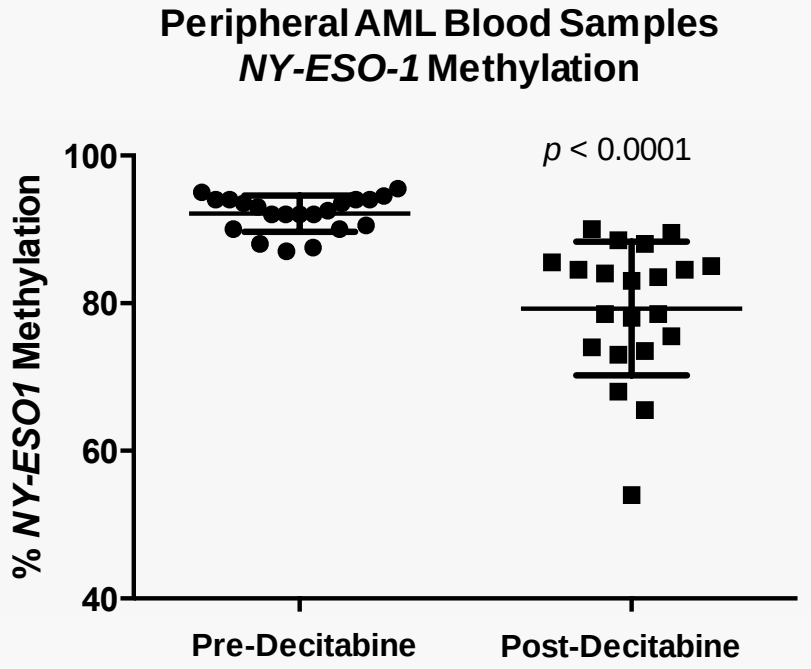
- BUT: Treatment with hypomethylating drugs might re-express CG genes (like *NY-ESO-1*) expanding vaccine applicability

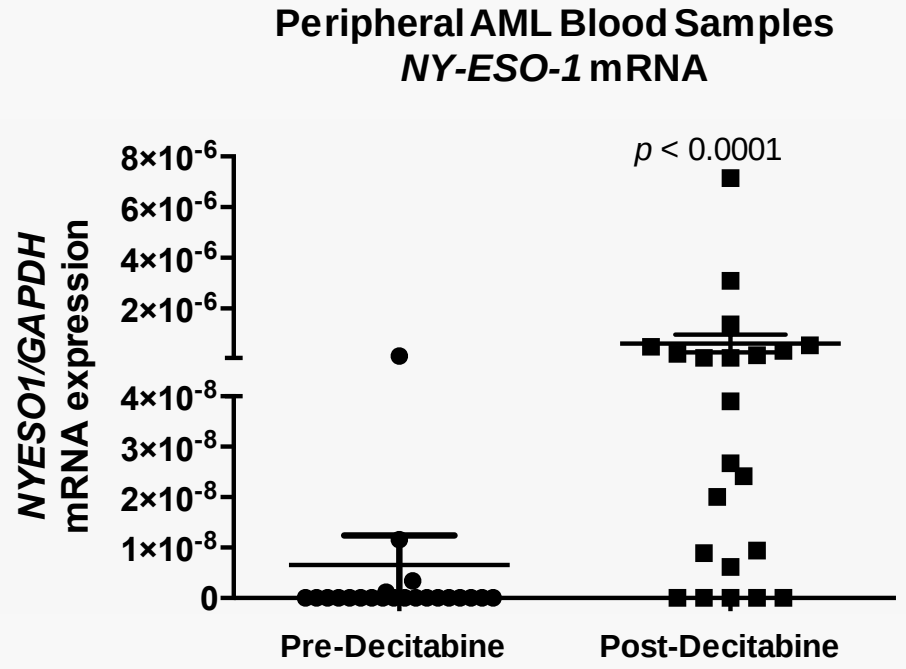
- AND: HMAs are standard of care for patients with myelodysplastic syndrome and AML

Following Dac, Primary AML samples Demonstrate Time-dependent Global Hypomethylation



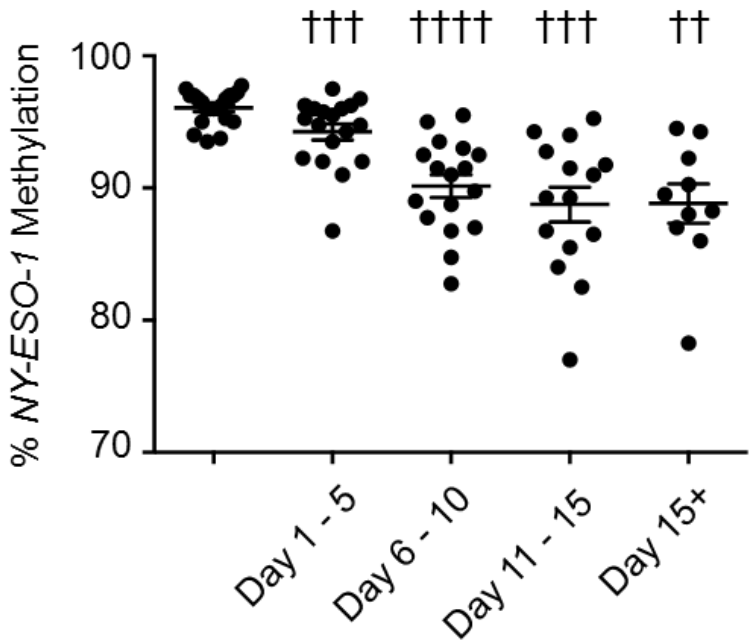
Following Dac, Primary AML Samples Demonstrate *NY-ESO-1* Hypomethylation, Gene Expression

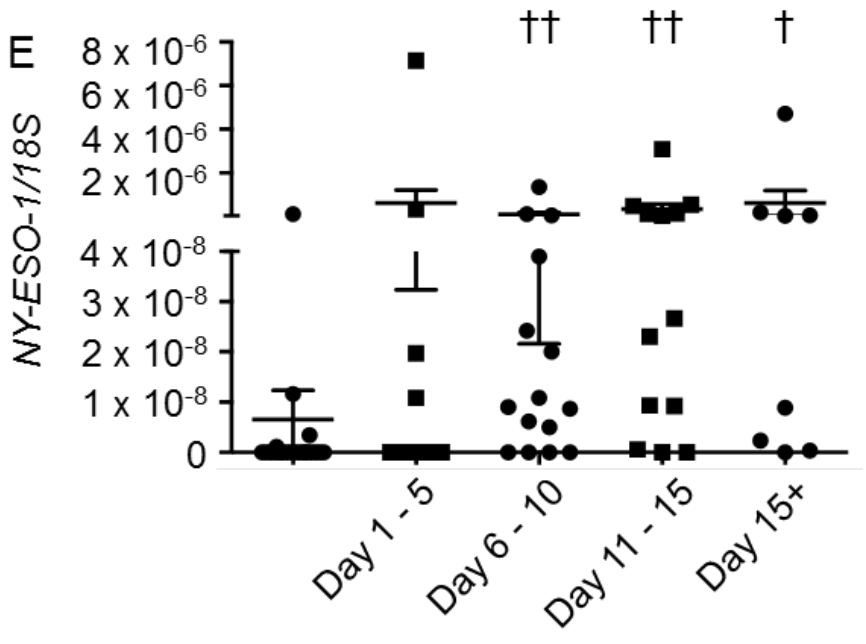




Srivastava et al. *Oncotarget 2016;7(11):12840-56.*

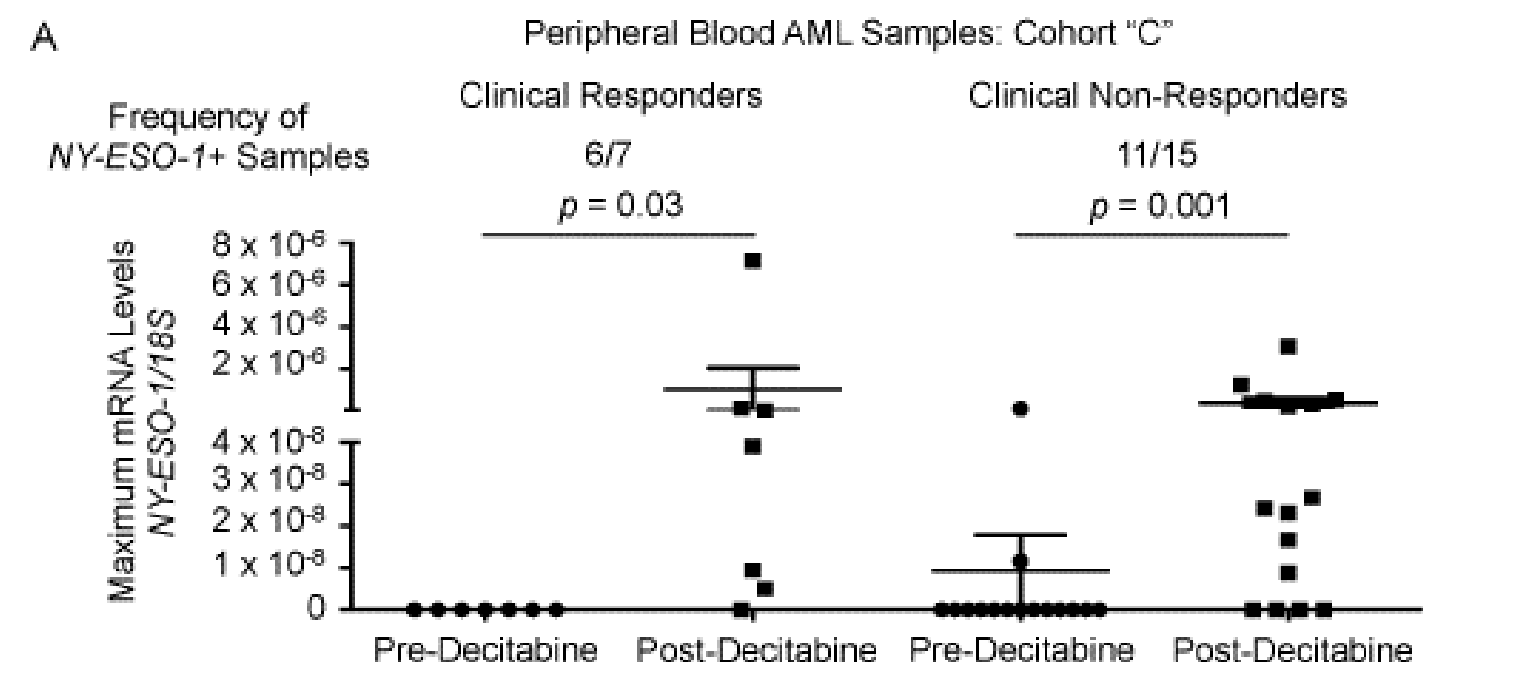
NY-ESO-1 Hypomethylation and Gene Expression are Time Dependent (n=22)





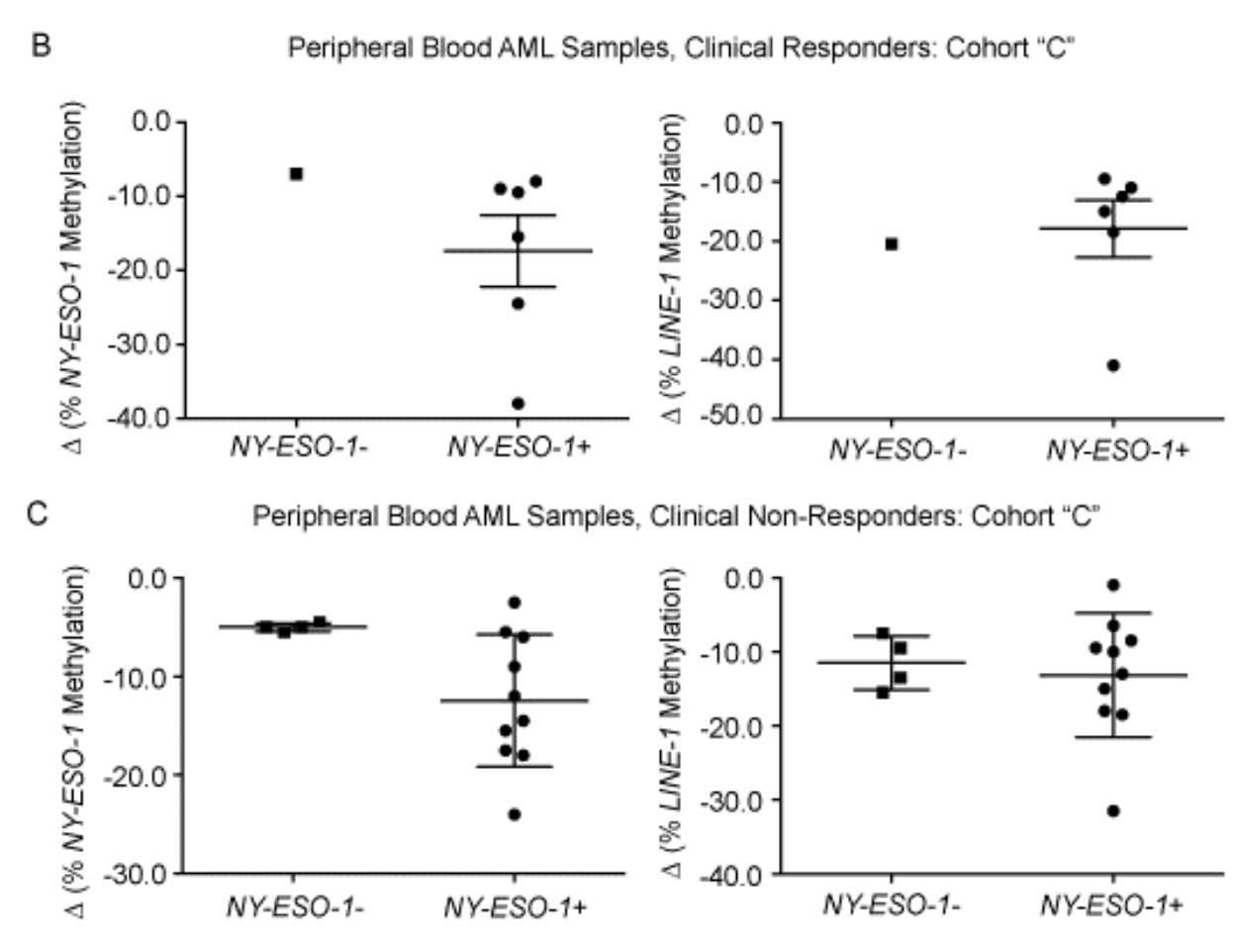
NY-ESO-1 Expression and Clinical Response

Clinical Response ≥ Hematologic Improvement



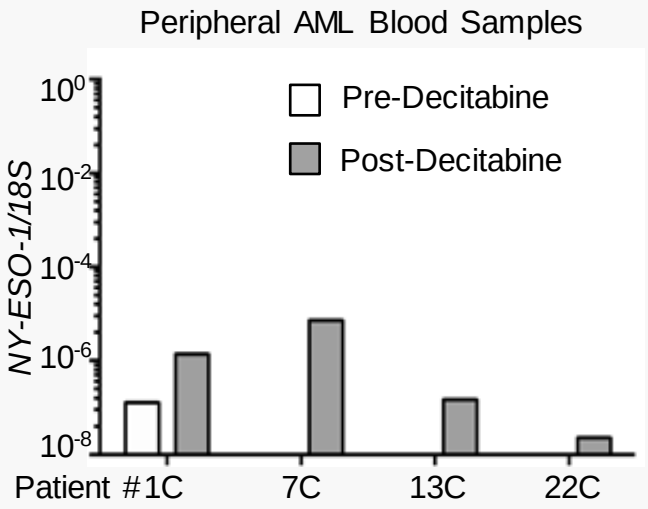
Srivastava et al. ***Oncotarget 2016;7(11):12840-56.***

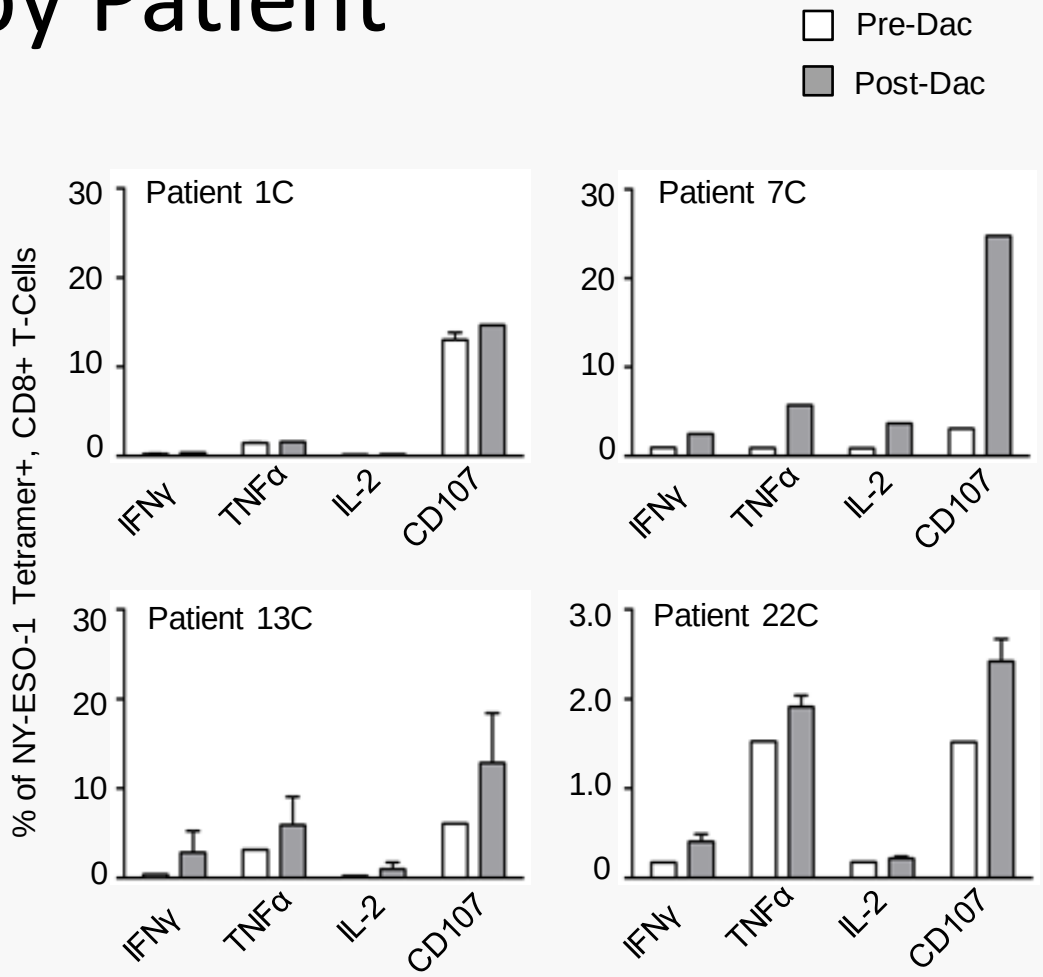
NY-ESO-1 Methylation and Clinical Response



Srivastava et al. *Oncotarget 2016;7(11):12840-56.*

Summary of Induced T-cell Responses by Patient

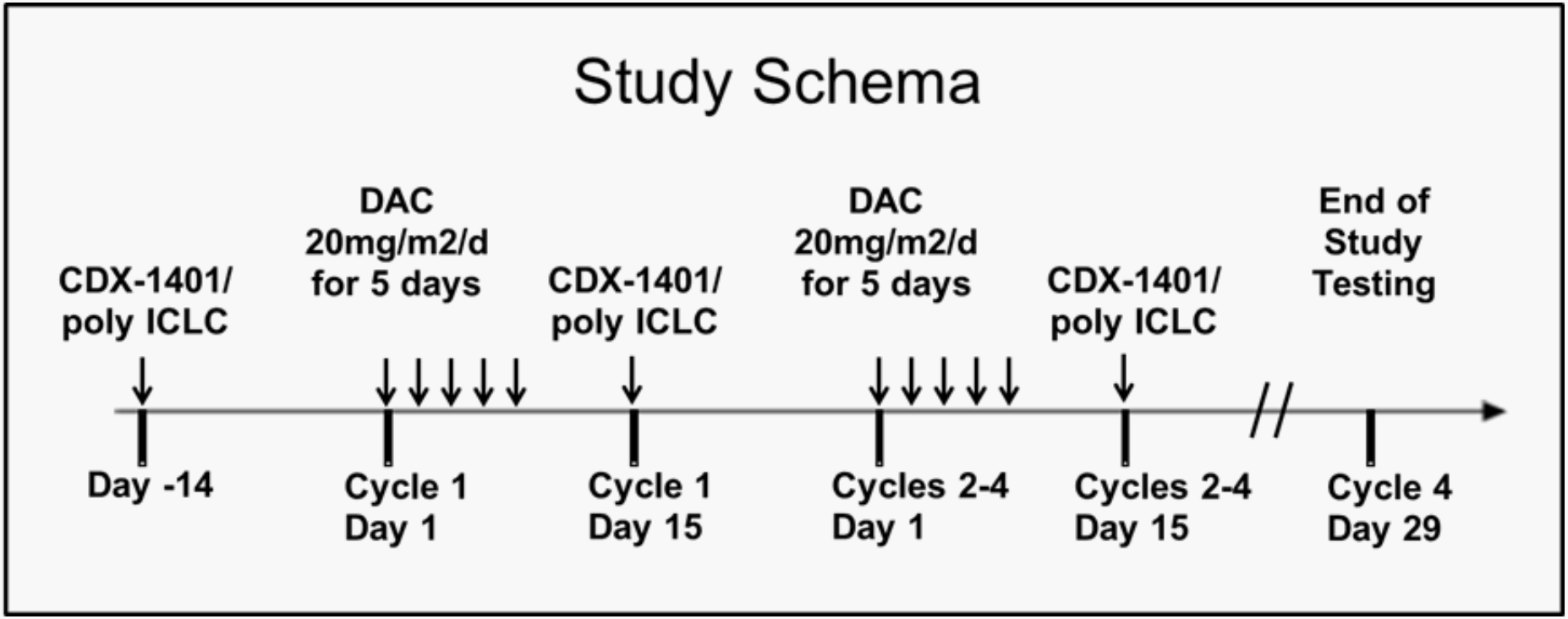




Retrospective Cohort Conclusions

- *NY-ESO-1* expression is induced in myeloid blasts from patients getting decitabine
- Protein expression/presentation sufficient to trigger a cytotoxic response in HLA compatible T-cells recognizing NY-ESO-1.

A Phase I Study of Decitabine in Conjunction with NY-ESO1 Vaccination in Pts with MDS or Low Blast Count AML



Vaccine: Celldex Therapeutics

- **Anti-DEC-205-NY-ESO-1 fusion protein (CDX-1401)**
  - Monoclonal Ab to DEC-205 on APCs fused to full length NY-ESO-1 protein (HLA unrestricted)
  - Phase I data in NY-ESO-1 expressing solid tumors
    - well tolerated
    - induces NY-ESO-1 $CD4^+$, $CD8^+$ T-cell, Ab responses.
- **Poly ICLC (stabilized poly-IC with poly-lysine**)
  - Viral mimic, activates innate immunity and Type I IFN
  - Immune-enhancer activates T, NK & DCs through induction of IFNs, ILs & TNF
  - Directly activates/targets DCs
    - w/o adjuvant, anti-DEC205-NYESO-1 could induce tolerance.

Dhodapkar MV et al. *Sci Transl Med* 2014; 6:232.

Study Specific Aims

- *Aim 1:* Determine the safety of vaccine + adjuvant in combination with Dac in patients with MDS/AML

- *Aim 2:* Determine the degree to which patients treated with Dac + vaccine develop NY-ESO-1 promoter hypomethylation and induce NY-ESO-1 mRNA and/or protein expression in circulating myeloid cells.

- *Aim 3:* Determine if vaccination in series with Dac can induce NY-ESO-1 specific cellular and/or humoral immunity.

Immunological Endpoints

- Measure NY-ESO-1 specific, IFNγ secreting CD4+ and CD8+ T-cells;
  - T0, D1, D15 each cycle, end of study using *in vitro* T-cell pre-sensitization-> ELISPOT for IFNγ production

- NY-ESO1 Specific Antibody (by ELISA) assessments
  - T0, D1, D15 each cycle and end of study.

- APC functional experiments pre-post Dac:
  - Ability of patient derived cultured APCs to activate donor NY-ESO1 specific T-cells
  - Ability of patient derived cultured APCs to produce an Allo response from healthy donor T-cells

- Baseline and post-dac flow cytometry for Treg subsets (CD127, CD45RA, CXCR3 and Helios) to determine immuneresponsive vs supressive phenotype

Safety

- 9 pts with MDS, median age 64y, have been enrolled.
- Safety cohort of 6 pts complete w/o unexpected toxicity
- AEs mostly Dac/disease related
  - cytopenias (predominantly grades 3/4),
  - elevated liver enzymes (grade 3),
  - fatigue (grade 2), edema (grade 2/3)
  - diarrhea (grade 1/2).
- Two patients withdrew from study early due to AEs:
  - 1 w/ h/o MI developed in-stent restenosis and recurrent MI;
  - One suffered a terminal intracranial hemorrhage due to thrombocytopenia (Dac related)
- 3 pts enrolled to an expansion cohort with no additional safety signals

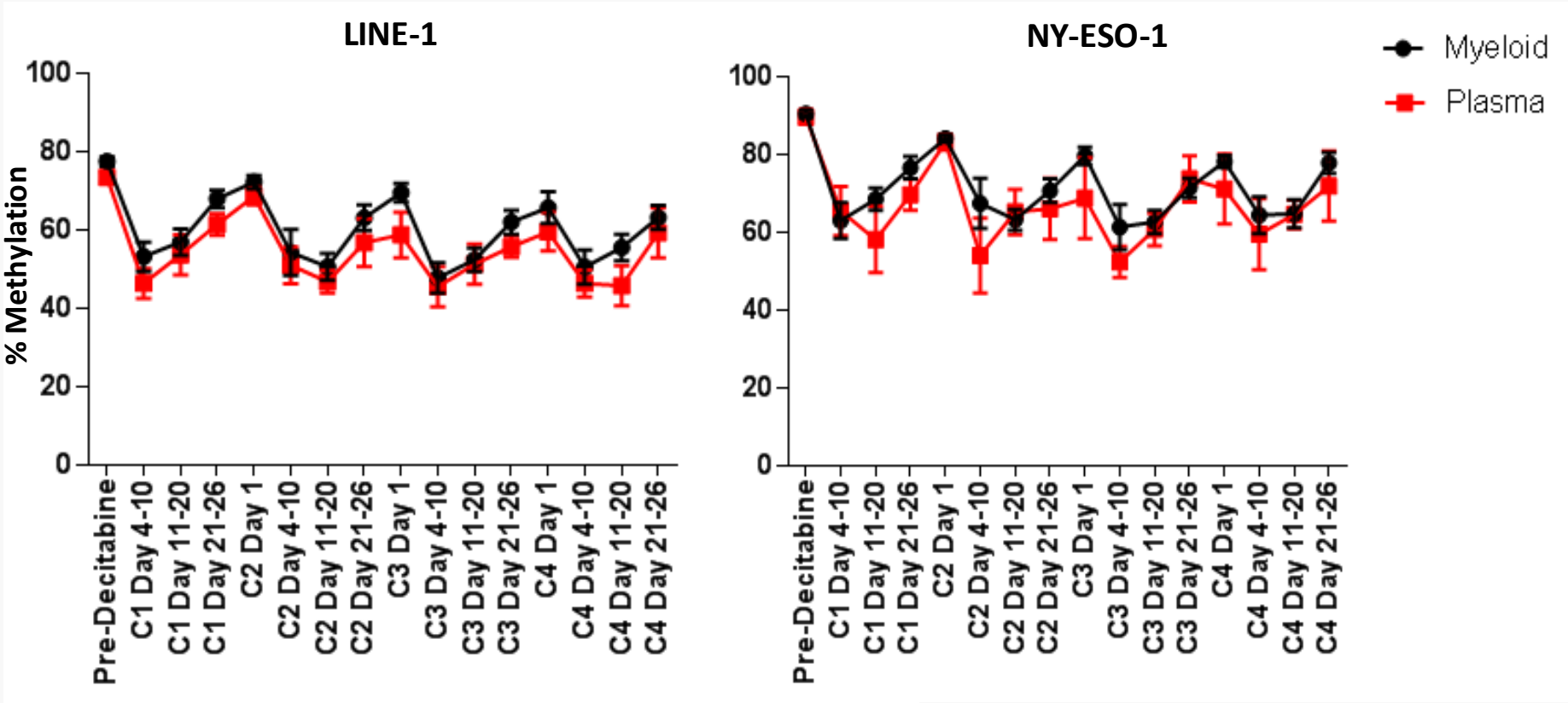
Demographics

Cohort Size	**n=9**
Age	**64 (57-71 yr)**
Male	**5 (56%)**
Female	**4 (44%)**
Diagnosis	**2 AML (22%); 7 MDS (88%)**

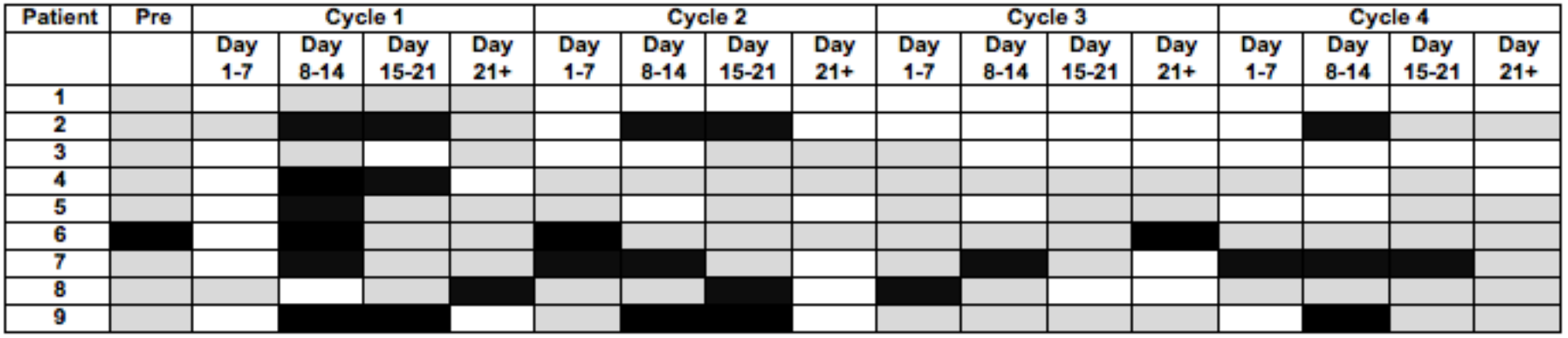
Safety

	All Grades	Grade ≥3
Cytopenias		
Anemia	**5**	**4**
Thrombocytopenia		**6**
Neutropenia		**6**
Hyperbilirubinemia	**5**	**1**
LFT Elevation	**8**	**0**
Diarrhea	**4**	**0**
Fatigue	**4**	**0**
Edema	**4**	**1**

Global, Target Specific Methylation in Peripheral Blood Compartments: Serially Sampled Patients (n=9)



NY-ESO-1 Expression in Myeloid Cells During HMA Therapy



Patient	Pre	Cycle 1				Cycle 2				Cycle 3				Cycle 4			
		Day 1-7	Day 8-14	Day 15-21	Day 21+	Day 1-7	Day 8-14	Day 15-21	Day 21+	Day 1-7	Day 8-14	Day 15-21	Day 21+	Day 1-7	Day 8-14	Day 15-21	Day 21+
1																	
2																	
3																	
4																	
5																	
6																	
7																	
8																	
9																	

Black = NY-ESO-1 Expression
Gray = No expression detected
White = ND

Immune Response

Patient	Antibody Titer		CD4 response		CD8 response		NY-ESO-1 expression	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	-	-	+ (1)	- (0)	- (0)	++ (3)	-	-
2	-	+	++ (2)	+++ (3)	- (0)	+ (1)	-	+
3	-	-	- (0)	+ (2)	- (0)	- (0)	-	-
4	-	-	- (0)	+ (2)	- (0)	+ (1)	-	+
5	-	-	- (0)	+ (1)	- (0)	- (0)	-	+
6	-	-	- (0)	+ (1)	- (0)	+ (2)	+	+
7	-	-	- (0)	+ (1)	- (0)	- (0)	-	+
8	-	-	- (0)	- (0)	- (0)	- (0)	-	+
9	-	++	+++ (1)	++++ (4)	- (0)	+++ (3)	-	+

-	<25	++	100-199	++++	>500
+	25-99	+++	200-499		

Intensity of response after subtracting background; (*) = number of epitopes recognized by T cells

Clinical Characteristics/Response

Pt	Dx	Age	Karyotype	IPSS Score	IPSS-R	Best Response	LTFU
1	RAEB-2	56	Complex; >3 abnormalities	High	V. High	CR	Died in CR from GVHD
2	RAEB-1	63	Complex; >3 abnormalities	Int-2	V. High	SD	Died from GVHD with active disease
3	RAEB-1	62	Complex; 3 abnormalities	Int-2	V. High	HI	Died from stroke
4	RAEB-2	65	2 abnormalities including del(20q)	High	V. High	HI-P,HI-N	Died in CR from GVHD
5	RCMD	71	Normal	Int-1	High	PD	Died from AML progression
6	MDS/ AML	67	Normal	Int-2	Int	HI-P	Alive s/p Allo
7	RAEB-1	79	Normal	Int-1	Int	CR	Alive s/p 20 cycles decitabine
8	CMML-1	60	Normal	Int-1	Int	SD	Alive s/p Allo
9	RAEB-1	68	Normal	Int-1	Int	CR	Alive s/p cycle 18 decitabine

Phase 1 Conclusions

- Combination was well tolerated, No DLTs or unexpected adverse events

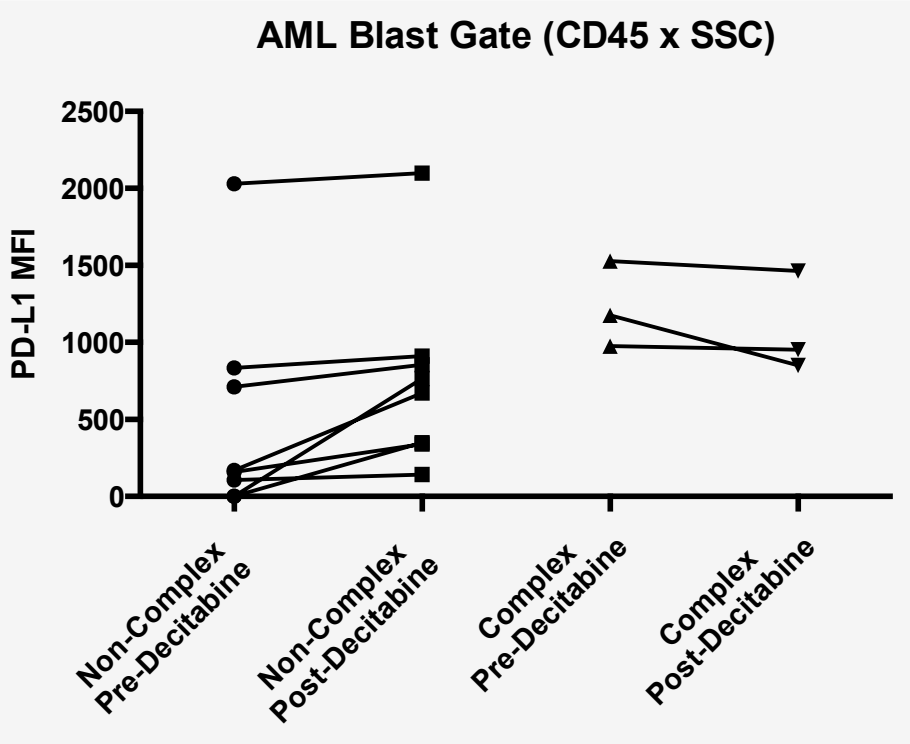
- Hypomethylation of *LINE-1*/*NY-ESO-1* observed in circulating myeloid cells, cell-free plasma DNA

- HMA treatment induces *NY-ESO-1* in circulating myeloid cells in MDS patients

- 2/9 developed NY-ESO-1 antibody response at EOS
- 7/9 patients with induced CD4+ T-cell Response
- 5/9 patients with induced CD8+ T-cell Response

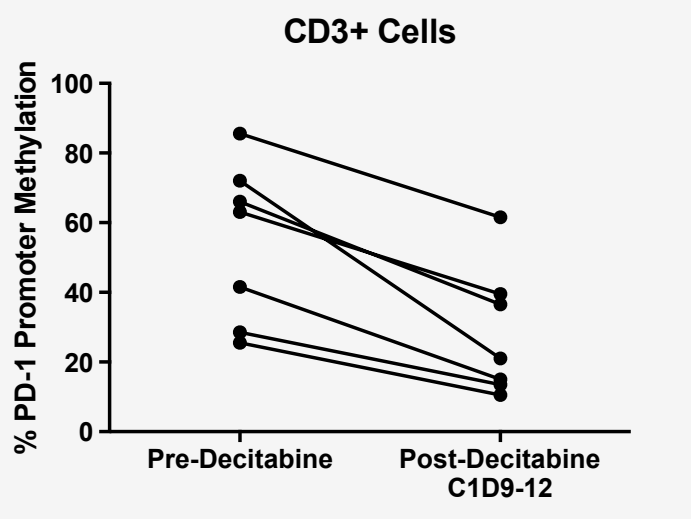
- Responses were less robust than observed in solid tumor studies (potential for combination with checkpoint blockade!)

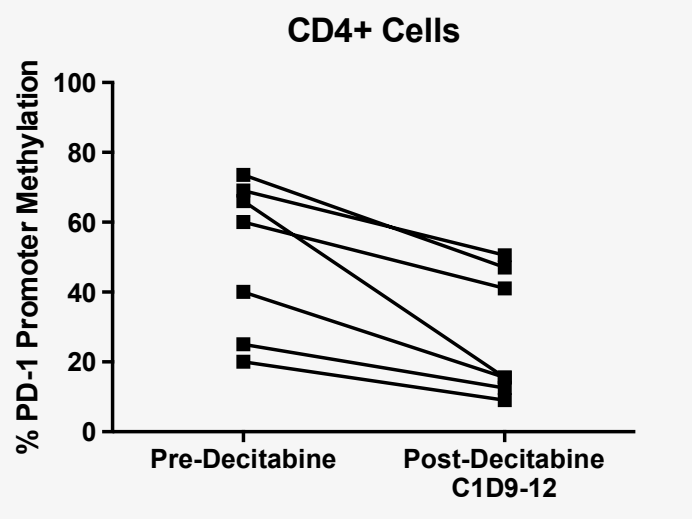
Expression of PD-L1 in AML Blasts

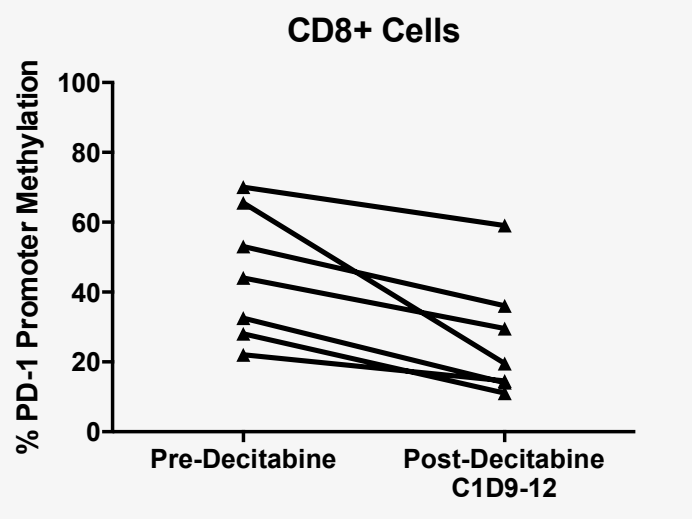


Complex: ≥ 3 Cytogenetic Abnormalities

PD1 Promoter is hypomethylated in AML T-cells following HMA therapy



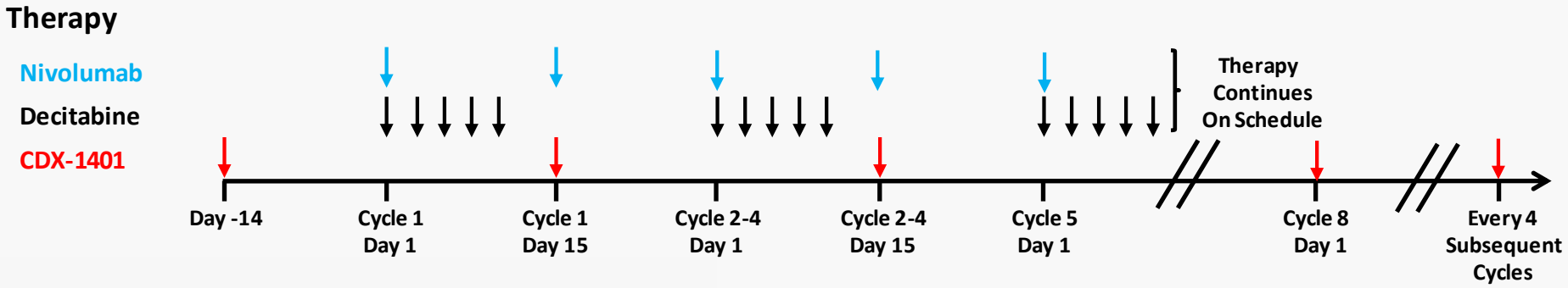




Nivo Project: AIMS

1) **Determine the safety of nivolumab in combination with decitabine and NY-ESO- 1 vaccination.**
2) **Evaluate the anti-NY-ESO-1 specific immune response following combination therapy with nivolumab, decitabine and NY-ESO-1 vaccination.**

A phase I/pilot study of DEC205mAb-NY ESO 1 fusion protein with adjuvant polyICLC in conjunction with 5-Aza-2'deoxycytidine (decitabine) and nivolumab in patients with MDS or low blast count AML



Decitabine 20mg/m2
CDX-1401: 1mg /poly ICLC 2mg
Nivolumab 3mg/kg

Eligibility

- Newly Diagnosed MDS/low blast count AML appropriate for HMA therapy
- ≥18y
- Non-transplant eligible
  - Due to age ≥75, comorbidity, personal choice or no donor
- Able to give informed consent

Study Objectives

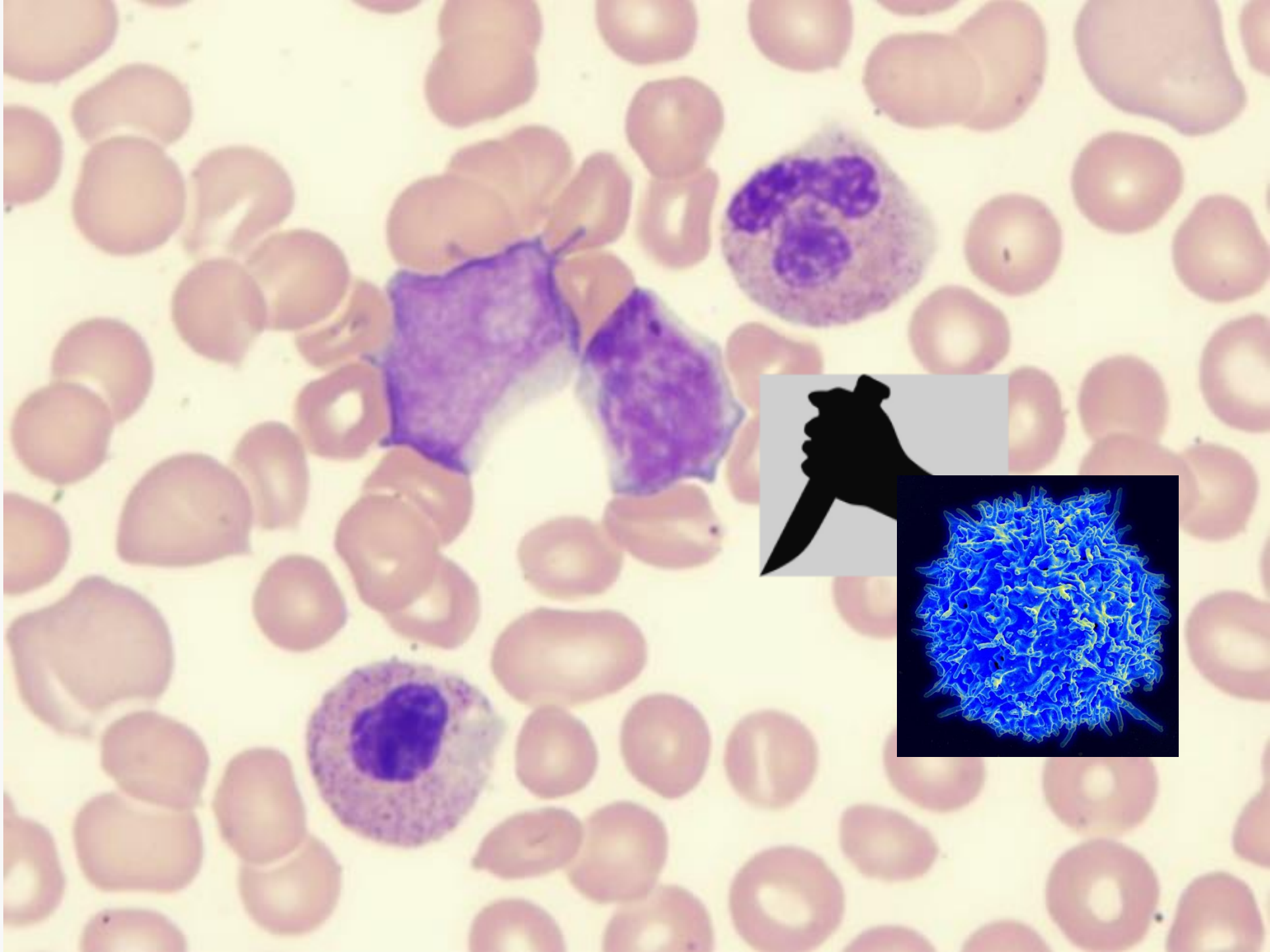
- **Primary**
  - Evaluate safety of combining NY-ESO-1 vaccine with decitabine 20 mg/m^2 intravenously and nivolumab 3 mg/kg
- **Secondary Objective**
  - Assess immune and molecular epigenetic responses following the three drug combination
- **Exploratory Objectives**
  - Determine response rate (Complete Response, Partial Response and Hematological Improvement) with the combination in order to provide descriptive characteristics.
  - Determine Overall Survival, Progression Free Survival and time to AML transformation (TTT) (for patients with MDS at diagnosis) enrolled on the study.

Correlative Assessments

- NY-ESO-1 specific, IFNγ secreting CD4+ and CD8+ T-cells; NY-ESO1 Specific Antibody (by ELISA) assessments ; Immune profiling by mass cytometry (Paul Wallace/Fluidigm collaboration)
- PD-1/PD-L1 expression in circulating T-cells/BM blasts
- NY-ESO-1 expression/ methylation in circulating myeloid cells, BM blasts at serial time points.
- Serial methylome/molecular assessment for clearance of malignant clones (Ken Figueroa collaboration).

Implications

- A comparison of cancer vaccine response with and without nivolumab in a relatively non-immunogenic tumor
- Provides a paradigm for induced target vaccination in combination with Nivolumab
  - *Significant impact for a broad range of solid tumors and translation to other inducible targets*
- Rapid readout due to disease cadence
- Potential for long term responses



Acknowledgements

Collaborators:

- Michael J. Nemeth PhD
- Adam R. Karpf PhD
- Kunle Odunsi MD, PhD
- Michael Lübbert MD, PhD
- James G. Herman MD
- Ken (Maria) Figueroa MD

Clinical Research Service:

- Justin Kocent
- Laurie Ann Ford MS
- Kerry Tocin BS
- Kemji Eke

Griffiths/Nemeth Labs:

- Pragya Srivastava PhD
- Ghadeer Fatani, MD
- Zachary Brumburger
- Christopher Ford

Funding Sources:

Rappaport Family Trust
Charles and Mary Bauer Memorial Fund
Sklarow Memorial Trust
Cancer Center Support Grant (RPCI) Funding
American Cancer Society (IRG)
Roswell Park Alliance Foundation
Astex Pharmaceuticals
Roswell Park Cancer Institute Startup Funds

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