

Epithelioid Sarcoma

Case Presentation

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January 2020

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Agenda

- Presentation of case
- Overview of Epithelioid sarcoma
- Molecular results (Impact panel, CeGat)
 - 37321-32 deletion
 - SWI/SWF and SMARCB1 loss
 - CREBBB
- Rationale for Tazemetostat Epigenetic Therapy
- Rationales for Immunotherapy
 - Rhabdoid tumor insights
 - HERV – perpetrator and protector
 - Hypomethylating therapy
 - Role for Vitamin C
 - Microbiome
- Metabolic therapy
 - Glutaminolysis
- Targeting Apoptosis: taurine?

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Social History

- 37, Indian descent, U.S. born
- Harvard educated
- Entrepreneur who created 1) non-profit company in Africa to lift women out of poverty by teaching them computer skills, 2) for profit cosmetics company
- married to Tassilo
- Life-long nonsmoker; no EtOH abuse
- Diet: mostly vegan; follows ketogenic diet since diagnosis

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Past Medical History	None	
Family History	No malignant diseases	
Allergy	mefloquine	

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HISTORY OF PRESENT ILLNESS	<p>The patient is a 37-year old otherwise healthy woman.</p> <ul style="list-style-type: none"> She presented with a 2 cm L. vulvar nodule of several months duration that was thought to be benign. It was excised by her local gynecologist on 3/1/2019. The pathology was reviewed at Stanford and characterized as an epithelioid sarcoma with a positive resection margin. There were 4 mitoses per 10 HPF with a Ki67 of 40%. The pancytokeratin and vimentin were positive, and P16 (HPV antigen) is described as having patchy positivity. Testing for INI1 expression by IHC was negative, confirming the diagnosis. 	
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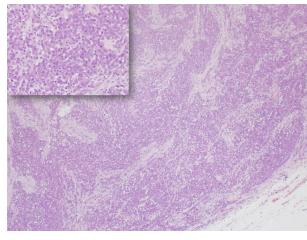
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Diagnosis of Epithelioid Sarcoma	<ul style="list-style-type: none"> Discovered 1970, (F Enzinger – Northwestern) Commonly misdiagnosed Mistaken for benign fibroma, granulomatous disease, carcinoma, sarcoma Presumed to arise from a mesenchymal precursor but follows a path of multi-directional differentiation (...¹due to being a disease of epigenetic dysregulation) Dual epithelial & mesenchymal markers presents confusion picture for pathologists: cytokeratin, epithelial membrane antigen, and CD34; as well as vimentin. Loss of nuclear protein INI1 (named for enhancing the DNA joining activity of HIV-1 integrase) expression due to SMARCB1 loss present in > 90% and is diagnostic. Rare: < 1% of STS; 1/5-10,000,000 – many physicians will never see a case in their careers. <p><small>Armaiz HB & Parwani AV. Archives of Pathol & Lab Med. 2009;133(5):814-819.</small></p>	
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Epithelioid Sarcoma

- Typically occurs between ages 20-40, median 27 (4-90 y.o.)
- Classified as proximal (trunk, perineal, pubic, genital) v. distal (extremities)
- Locally invasive, commonly metastasizes to lymph nodes and distant organs
- Prognosis
 - Favorable: young age at 1st diagnosis, female sex (75% survival rate vs 64% for men), and tumor <5 cm²
 - Unfavorable: older age, multifocal local disease, initial proximal limb or axial location, depth of invasion, high mitotic activity, necrosis, vascular invasion, and tumor size >10 cm². Histologic features such as pleomorphism, atypia, and infiltrative growth pattern predict poor prognosis.
- Metastatic disease occurs in 30-40%
- Chemotherapy resistant or poorly responsive as a rule
- Median survival of metastatic disease < 1 year



Arma H B & Parwan AV. Archives of Pathol & Lab Med. 2009;133(5):814-819.

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HPI, continued

- During the time waiting for a subsequent surgery in NY, the tumor grew back with a vengeance.
- 5/7/2019: radical L. vulvectomy, L. inguinal lymphadenectomy, omental free flap closure with vascular anastomosis, R. inguinal sentinel LN biopsy.
- Pathology:
 - 3.5 x 2 cm tumor regrew in the vulva between surgeries
 - Positive margins
 - + L. sentinel node: 0.4 cm tumor
- 5/13/19: CT chest/abdomen/pelvis and MRI of pelvis revealed *no evidence of metastatic disease*.
- 5/24/19: JP surgical drains removed
- 5/28/19: At followup visit after surgery tumor was noted to be growing back in L. groin

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Molecular Diagnosis (MSKCC Impact Panel)

Summary		1 mutation, 2 copy number alterations, no structural variants detected
MSI Status		MICROSATELLITE STABLE (MSS). See MSI note below. ¹
Tumor Mutation Burden		The estimated tumor mutation burden (TMB) for this sample is 0.9 mutations per megabase (m ² /Mb). The median TMB for all samples of patients with soft tissue sarcomas is 3.9 m ² /Mb and for patients with Soft Tissue Sarcoma is 1.1 m ² /Mb as of the date this report was issued.
Gene	Type	Alteration
Mutations		Location
CREBBP	Missense Mutation	M779V (c.2334A>G)
Copy Number Alterations		exon 13
SMARCB1	Whole gene	Deletion
MAP3K14	Whole gene	Loss

TMB: one of the lowest of all human cancers; therefore not expected to elicit an immune response...

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Whole Exome NGS (CeGaT)*

Tumor mutational burden (TMB): low (0.7 variants/megabase)
Microsatellite instability: no evidence for MSI (based on a prediction from NGS data)
Copy number alterations: evaluable with certain limitations
Translocation: no evidence of therapy relevant translocations/fusions
Germline variants: no evidence of likely disease causing or therapy relevant germline variants.

No Driver mutation identified.

Chromosomal region	Functional category	Variant	Copy number	Affected genes with potential therapeutic relevance
chr17 41791652-46807594	probably heterozygous deletion	q-arm, partial	1	-
chr22 22871570-24407765	probably heterozygous deletion	q-arm, partial	1	SMARCB1

*The quality of the nucleic acids did not allow for transcriptomic analyses.

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Neoepitope prediction based on HLA

No	Peptide	Gene and Coding_Info	NAF DNA	HLA allele
1	MVCWLSAK	WDR81_NM_001163809:c.C3688T>p.R1230C	0.17	HLA-A*11:01, HLA-A*68:01
2	AVYADLLR	COPS7A_NM_001164095:c.G418C>p.V140L	0.17	HLA-A*11:01, HLA-A*68:01
3	LTFTEAQR	HAPLN4_NM_023002:c.G535A>p.A179T	0.20	HLA-A*11:01, HLA-A*68:01
4	NMVGSAHTNNM	CREBBP_NM_004380:c.A2326G>p.M776V	0.20	HLA-B*15:02
5	KSPPLSTLPL	BCL11B_NM_138576:c.C1220T>p.P407L	0.19	HLA-C*15:02
6	RMPQPPNMVGAGTNMM	CREBBP_NM_004380:c.A2326G>p.M776V	0.20	Class II

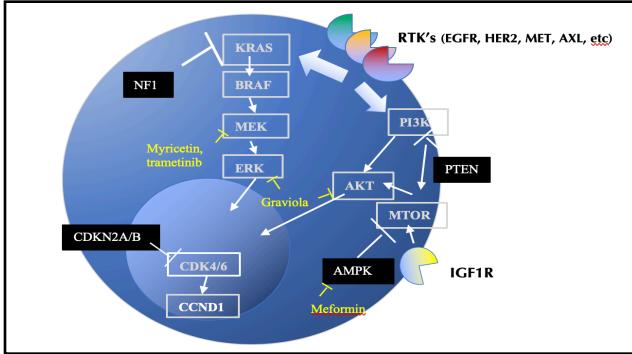
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17q21.32 deletion

- The deleted segment of 17q contains two tumor suppressor genes, ITGB3 and miR-153
 - ITGB3 has been implicated as a tumor suppressor in ovarian cancer, but no therapeutic modalities have emerged to address its absence.
 - miR-152 has been studied in breast cancer. Its loss results in the over-expression of UGF1R and IRS1 resulting in the activation of MTOR and MAPK pathways.*

* Xu Q, et al. J Mol Cell Bio, 2013, 5(1), 3-13.

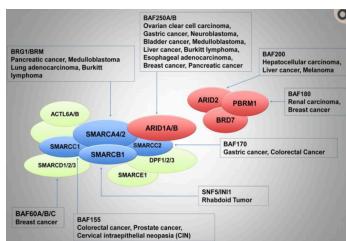
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SWI/SWF and Cancer SMARCB1 Context

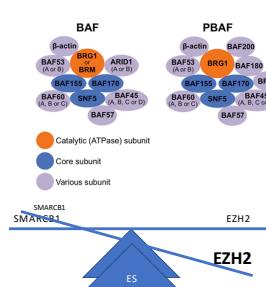
- Since 2010, WGS and WES demonstrated that chromatin dysregulation is linked to cancer development.
 - We now know that there are 15 subunits of SWI/SWF encoded by 29 genes that make up the chromatin remodeling genes and determine which genes are marked for transcription, which marked for silence.
 - Mutations involving SWI/SWF are implicated in > 20% of human cancers.



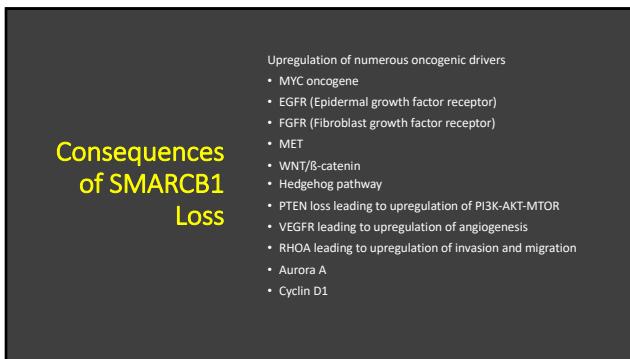
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SMARCB1 (SNF5/INI1/BAF47)

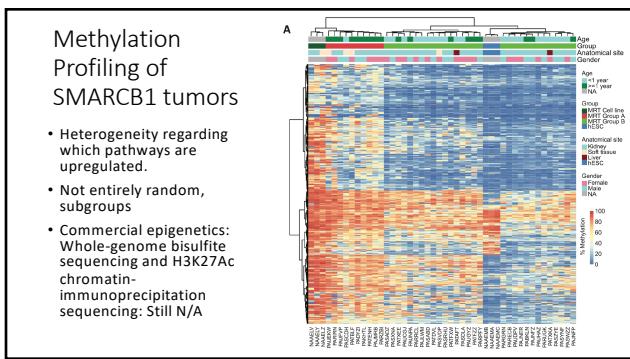
- Located on chromosome 22q11
 - Core member of the SWI/SWF chromatin(or nucleosome) BAF and PBAF remodeling complex.
 - functions as an acetylase that turns on a wide variety of genes. Hence, SMARCB1 deficient cancers tend to be deacetylated cancers where genes responsible for growth control are switched into the "off" position.
 - SWI/SNF complex is counter balanced by the Polycomb repressor complex (PRC2) whose dominant member is EZH2. EZH2 which is responsible for trimethylation (H3K27me3) that represses gene transcription



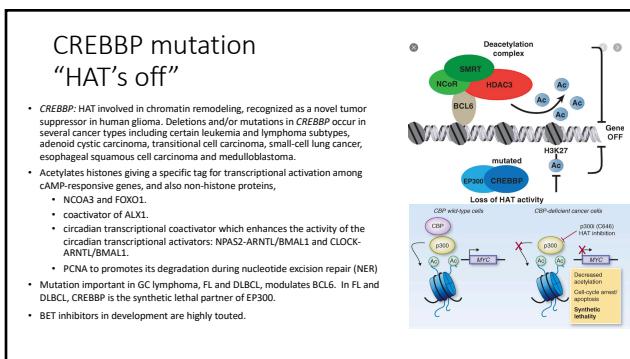
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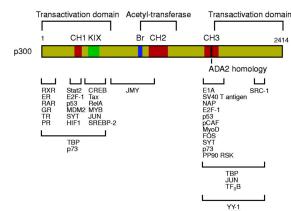
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CREBBP c. 2326 A>G

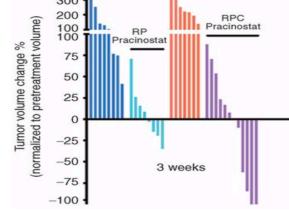
- This specific mutation affects the transactivation domain and affects binding to a wide variety of proteins and transcription factors, including:
 - p53
 - Fos
 - JUN
- The binding of numerous DNA viruses (including adenovirus, HPV, and EBV) to the transactivation domain inactivates CREBBP.
- CREBBP loss is associated with epithelial-mesenchymal transition (EMT), stem cell phenotype which is chemotherapy resistant, loss of CDH1 expression (which mediates cell-cell contact inhibition), and upregulation of transcription targets that drives cell proliferation by activating the cell cycle.



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Therapeutic proposal

- Normally the balance of acetylation and methylation is maintained by histone deacetylases.
- (HDAC) which remove acetyl groups from H3K27 and other methylases. Hence, blocking HDAC is HDAC inhibition, would increase acetylation to overcome loss of acetylation caused by CREBBP.
- Laboratory study of HDACi show efficacy and preferential sensitivity in cancer with CREBBP mutation.
- While HDACi have not been studied in combination with other strategies, valproic acid is a prototypical HDAC inhibitor which has been used with chemotherapy and is a reasonable choice in a patient with both SMARCB1 and CREBBP loss.



Hellwig M, et al. Cancer Gene Therapy, 2019,
doi:10.1038/s41417-019-0099-5

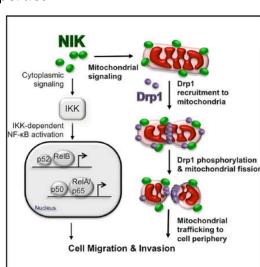
Deshui J, et al. Cancer Discovery, 2018, 1422-37.

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MAP3K14 (NIK) Loss Oncogene and tumor suppressor gene properties

- NIK-inducing kinase (NIK) is the central activator of the non-canonical NF- κ B pathway promotes tumorigenesis through its ability to regulate cell proliferation and survival.
- NIK regulates the velocity and anterograde movement of mitochondria toward the periphery of migrating cells, suggesting that increased fission, mitochondrial motility, and cell invasion are functionally linked.
- On the other hand, NIK signaling attenuates the development of colorectal cancer through activation of the non-canonical NF- κ B signaling cascade which leads to significantly reduced *CCL19*, *CCL21*, *CXCL12*, and *CXCL13* expression in CRC patients that normally protect GUT from carcinogens.
- NIK loss leads to altered trafficking of leukocytes that results in eosinophilic esophagitis.

Irving C Allen, et al. J Immunol May 1, 2017, 198 (1 Supplement) 197-6
Jung JU, Ravi S, Lee DW, et al. Curr Biol. 2016;26(24):3288-3302.



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ASCO June 2, 2019

Safety and efficacy of tazemetostat, a first-in-class EZH2 inhibitor, in patients with epithelioid sarcoma (ES) (NCT02601950)

A phase 2 study of an investigational drug

Silvia Stacchetti¹, Patrick Schulteck², Robin L. Jones³, Mark Agius⁴, Victor Vilasboas⁵, Thierry Jahan⁶, Tom Wei-Wu Chen⁷, Antoine Italiano⁸, George D. Demetri⁹, Gregory M. Cole¹⁰, Roshini Chugh¹¹, Steven Attia¹², Alisa Gupta¹³, Elisabeth T. Loggers¹⁴, Brian A. Van Tine¹⁵, Laura Sierra¹⁶, Jay Ying¹⁷, Anand Rajarethnam¹⁸, and Mirjal Gounder¹⁷

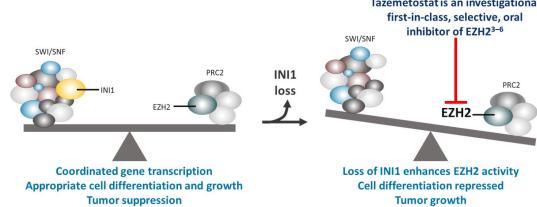
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► EPITHELIOID SARCOMA (ES), DISEASE OVERVIEW

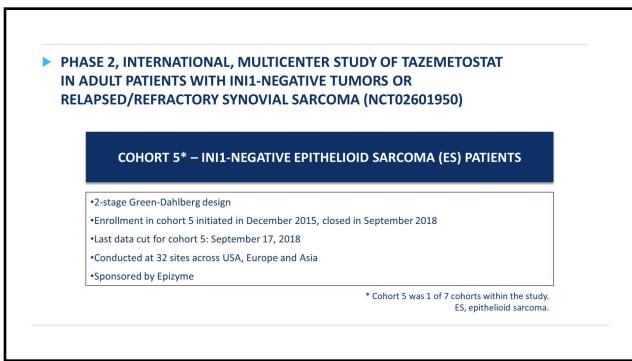
ULTRA RARE SARCOMA	INCIDENCE: FEMALES, 0.01–0.02/100,000; MALES 0.04–0.05/100,000 ^{1–3}
PATHOLOGY/ MOLECULAR PROFILE	TWO PATHOLOGICAL SUBTYPES: DISTAL AND PROXIMAL ES INI1 LOSS OCCURS IN >90% OF PATIENTS ⁴
YOUNG PATIENT POPULATION	PEAK ONSET OF DISTAL ES: 20–30 YEARS PEAK ONSET OF PROXIMAL ES: 30–40 YEARS ⁴
POOR PROGNOSIS	PATIENTS WITH METASTATIC DISEASE: 5-YEAR OVERALL SURVIVAL (OS) 0% ⁴
FEW MEDICAL TREATMENT OPTIONS	MEDIAN PROGRESSION-FREE SURVIVAL (PFS) OF ANTHRACYCLINE-BASED CHEMOTHERAPY: 3 MONTHS ⁵ MEDIAN PFS OF GEMICITABINE-BASED CHEMOTHERAPY: 8 MONTHS ⁵ NO SPECIFIC TARGET TREATMENT AVAILABLE

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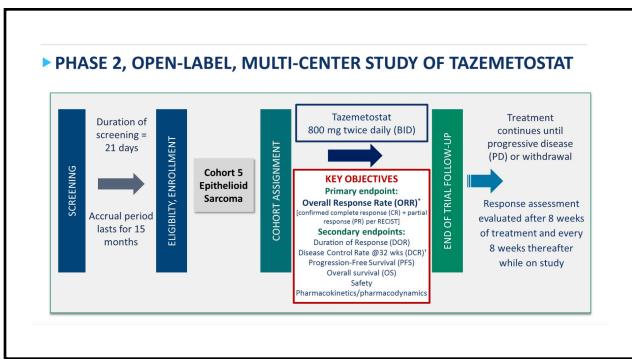
► LOSS OF INI1 CREATES AN ONCOGENIC DEPENDENCY ON ENHANCER OF ZESTE HOMOLOG 2 (EZH2)



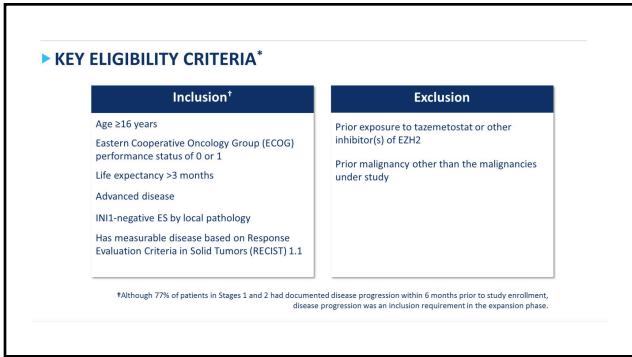
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BASELINE DEMOGRAPHICS, INI1-NEGATIVE ES PATIENTS (COHORT 5; N=62)	
Characteristic	Patients (N=62*)
Age (years), mean (Standard Deviation)	37 (15.1)
Male/female, n (%)	39 (63)/23 (37)
Subtype, n (%)	Proximal 27 (44) Distal 31 (50) Missing categorization 4 (6)
Stage at diagnosis,* n (%)	I 2 (3) II 7 (11) ≥III 44 (71)
Characteristic	Patients (N=62*)
Progressive disease prior to study entry, Yes/No, n (%)	59 (95)/3 (5)
Lines of prior anticancer therapy, n (%)	Median (range) 0 24 (39) 1–3+ 38 (61)
ECOG Status, n (%)	0 36 (58) 1 21 (34) 2 5 (8)

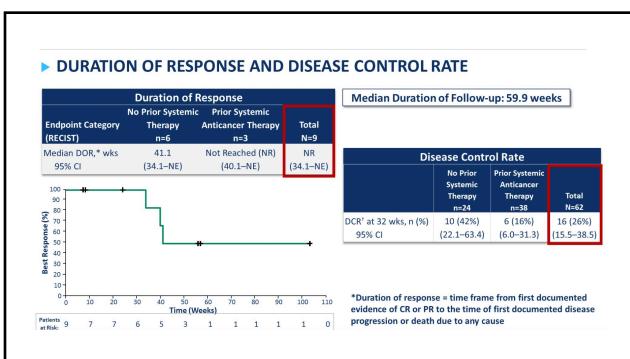
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► MOST FREQUENTLY OCCURRING ADVERSE EVENTS (AEs)					
Category, n (%)	All Treatment-Emergent AEs (TEAEs) (N=62)		Treatment-related AEs (N=62)		Grade ≥3
	Any Grade	Grade ≥3	Any Grade	Grade ≥3	
NON-HEMATOLOGIC AEs					
Fatigue	24 (39)	1 (2)	17 (27)	1 (2)	
Headache	22 (35)	0	17 (37)	0	
Cancer pain	20 (32)	3 (5)	3 (5)	0	
Decreased appetite	16 (26)	3 (5)	10 (16)	1 (2)	
Vomiting	15 (24)	0	10 (16)	0	
Constipation	13 (21)	0	5 (8)	0	
Headache	11 (18)	0	4 (6)	0	
Cough	11 (18)	0	0	0	
Diarrhea	10 (16)	0	8 (13)	0	
Weight decreased	10 (16)	4 (6)	4 (6)	2 (3)	
Dyspnea	8 (13)	4 (6)	0	0	
Pleural effusion	7 (11)	4 (6)	0	0	
HEMATOLOGIC AEs					
Anemia	10 (16)	8 (13)	6 (10)	4 (6)	
Thrombocytopenia	2 (3)	0	0	0	
Lymphopenia	1 (2)	0	1 (2)	0	
Neutropenia	0	0	0	0	

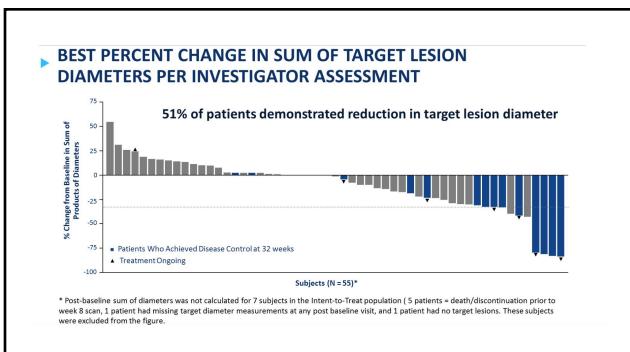
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► PRIMARY STUDY ENDPOINT: OBJECTIVE RESPONSE RATE (ORR) PER RECIST			
Endpoint Category (RECIST), n (%)	No Prior Systemic Therapy (n=24)	Prior Systemic Anticancer Therapy (n=38)	Total (N=62)
ORR (CR+PR)* 95% CI	6 (25%) (9.8–46.7)	3 (8%) (1.7–21.4)	9 (15%) (6.9–25.8)
CR	0	0	0
PR	6 (25%)	3 (8%)	9 (15%)
SD	15 (63%)	20 (53%)	35 (56%)
PD	2 (8%)	11 (29%)	13 (21%)
Not evaluable	1 (4%)	4 (10%)	5 (8%)

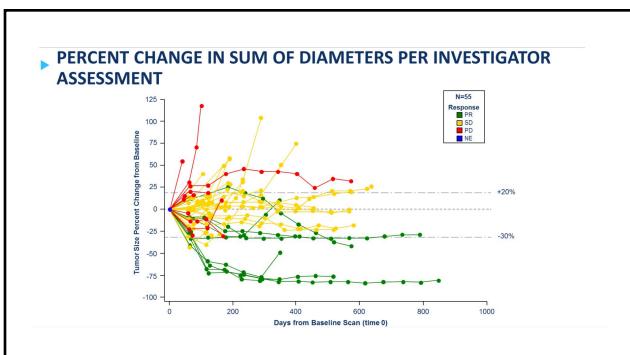
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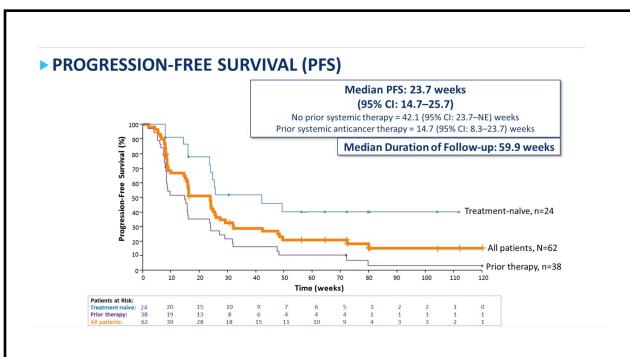
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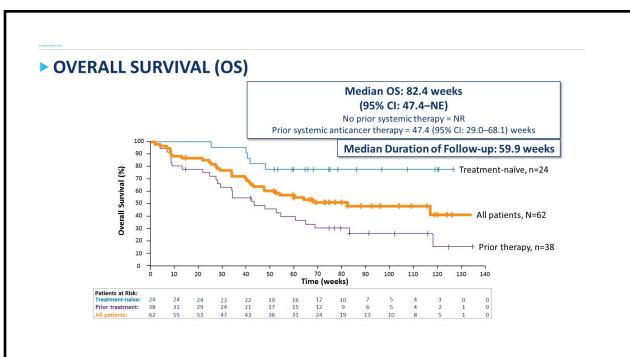
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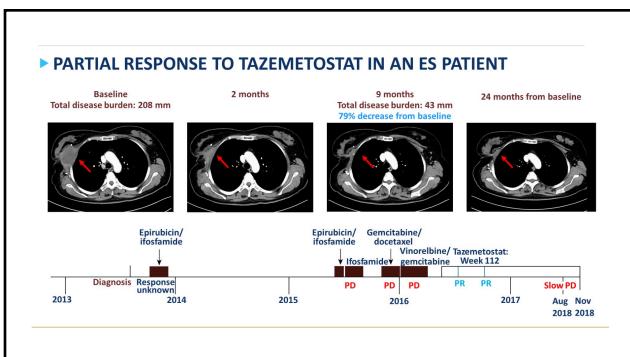
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SUMMARY

FIRST PROSPECTIVE STUDY CONDUCTED IN EPITHELIOD SARCOMA

TREATMENT WITH TAZEMETOSTAT, AN INVESTIGATIONAL, FIRST-IN-CLASS ORAL EZH2 INHIBITOR, ACHIEVED

- AN ORR BY RECIST IN 15% OF ALL PATIENTS
- A DECREASE IN TUMOR SIZE IN 51% OF ALL PATIENTS
- DURABLE RESPONSES. AT A MEDIAN FOLLOW-UP OF 59.9 WEEKS, THE MEDIAN DOR WAS NOT REACHED
- A MEDIAN PFS OF 23.7 WEEKS, WITH 21.3% PATIENTS PROGRESSION-FREE AT 1 YEAR.
- A MEDIAN OS OF 82.4 WEEKS

TAZEMETOSTAT WAS GENERALLY WELL TOLERATED WITH NO TREATMENT-RELATED DEATHS AND <2% DEFINITIVE DISCONTINUATIONS

TAZEMETOSTAT, IF APPROVED, REPRESENTS A POTENTIAL THERAPEUTIC OPTION IN INI1-NEGATIVE ADVANCED ES PATIENTS

- EPIZYME RECENTLY SUBMITTED A NEW DRUG APPLICATION (NDA) TO THE U.S. FOOD AND DRUG ADMINISTRATION (FDA) FOR ACCELERATED APPROVAL OF TAZEMETOSTAT FOR THE TREATMENT OF PATIENTS WITH METASTATIC OR LOCALLY ADVANCED EPITHELIOD SARCOMA NOT ELIGIBLE FOR CURATIVE SURGERY

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ACKNOWLEDGMENTS

Epizyme thanks all sites, study coordinators, and most of all, the patients, caregivers, and families that have contributed to the study

The following sites were instrumental in enrolling patients in this study:

Dana-Farber Cancer Institute, Fondazione IRCCS Istituto Nazionale Tumori, Fred Hutchinson Cancer Research Center, Institut Bergonie, Massachusetts General Hospital, Mayo Clinic in Florida, Memorial Sloan Kettering Cancer Center, Michigan Medicine Comprehensive Cancer Center, National Taiwan University Hospital, Northwestern Memorial Hospital, Princess Margaret Hospital, The Royal Marsden Hospital and Institute for Cancer Research, University Hospital Leuven, University of California, San Francisco, University of Colorado, Washington University in St. Louis School of Medicine

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Regulatory Status of Tazemetostat

Tazemetostat Submitted for FDA Approval

Oncology Drug Advisory Committee vote unanimously in favor of approval for ES.

31 May 2019

25 July 2019

19 Dec. 2019

23 Jan. 2020

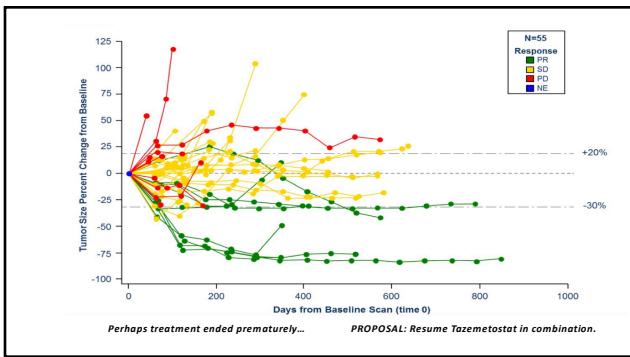
Epizyme announced that the FDA granted Priority Review for the New Drug Application (NDA)

Prescription Drug User Fee Act target action

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HPI, continued

- Gemcitabine x 3 doses was administered but associated with pneumonitis necessitating generous corticosteroid doses which compromises T cells and continuation of GEM.
- One dose of Doxil was administered without benefit.
- Progressive metastatic disease ensued in the chest, liver, and bone during November and December.
- L. neck radiotherapy
- Pazopanib x 4 days
- Chest radiotherapy was administered.
- Mid-December: Anti-PD-1/L1 immunotherapy with nivolumab (Opdivo™) was instituted based on a recent paper in the pediatric neuro-oncology pertaining to rhabdoid tumors (To be discussed.)
- Ultimately, disease progression produced superior vena cava syndrome, refractory pain, falling platelet count (45,000), and additional AIM chemotherapy was administered with reservations.
- Fortunately, Leila is having an immediate response to the treatment, albeit with severe mucositis. She is eligible to get 1 or at most 2 more cycles of aim.*

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Rationale for Immunotherapy

Rhabdoid Tumors - Sister disease of Epithelioid sarcoma

- First described as distinct entity in 1980's, but not recognized by WHO until 1993.
- Comprise 15% of all infant cancers in US and UK, (including brain tumor and extracranial tumors of the kidney and neural crest)
- Driven by bi-allelic loss of SMARCB1 (the sister disease of ES), rarely SMARCA4
- One of the lowest mutation burdens of all cancers and has a predominantly diploid and comparably stable genome. It is an epigenetic cancer.

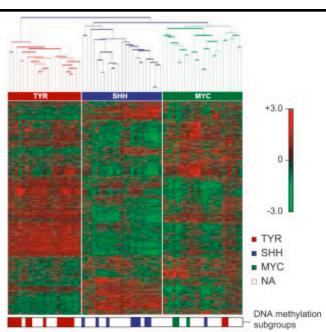
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Epigenetic subtypes of Rhabdoid tumors

Unsupervised Hierarchical Clustering Analysis

Biologically heterogeneous cancers:

- ATRT-TYR: tyrosinase over-expressing, over expression of melanocytic markers, usually infratentorial; a characteristic of this subgroup is the overexpression of many genes involved in oligogenesis and ERBB2, VEGFR and MET (potentially responsive to radiation and MEK inhibitor, known for CD8 infiltration).
- ATRT-SHH: supra-and infratentorial, over-expression of Hh pathway with high levels of GLI2, PTHC (potentially responsive to various targeted therapies), CDKN2A, MYCN, CDK6, and NOTCH1 (ASCL1, DLL3, HES 5/6)
- ATRT-MYC: over-expression of MYC and HOX gene cluster mostly supra-tentorial, ERBB2 over-expression



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Some RT's trigger immune response

- Despite low TMB, they are paradoxically characterized by*:
 - CD8+ T-cell invasion
 - expression multiple of checkpoint receptors (PDCD1/PD-1, HAVCR2/TIM-3, LAG3, ENTPD1/COD9TFRS9/4-1BB, and TIGIT) associated with T cell exhaustion (Chronic T cell stimulation)
 - expression of multiple IFN-regulated genes such as IFIT1 and ISG15, and a high enrichment in the interferon- α (IFN- α) response signature;
 - MYC subtype has much more robust T cell invasion than SHH subtype
- Immunogenicity linked to expression of human endogenous retrovirus...!*

DIAGNOSTIC PROPOSAL

- Obtain transcriptomic analysis to determine molecular subtype and upregulated pathways
- Perform Immunomic profiling to determine immune evasion strategies (Alacris Theranostics).

*Leruste et al., Clonally Expanded T Cells Reveal Immunogenicity of Rhabdoid Tumors, *Cancer Cell* (2019)

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Human endogenous retrovirus (HERV)

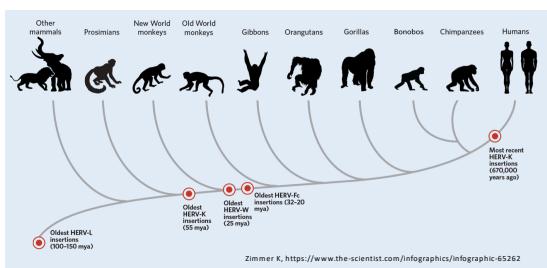
"If Charles Darwin reappeared today, he might be surprised to learn that humans are descended from viruses as well as from apes."

-Robin Weiss

Weiss. The discovery of human retrovirus. *Retrovirology*, 2006, 3:67

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HERV-K was acquired by humans 670,000 years ago

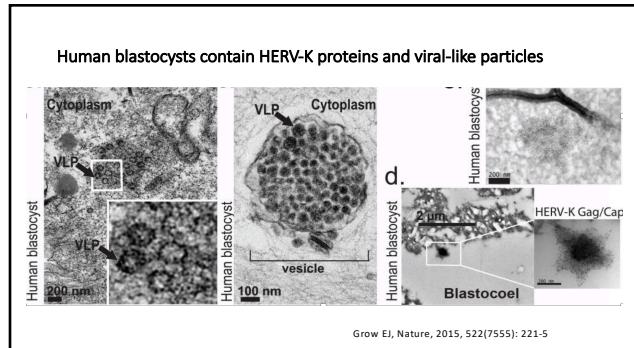


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HERV Basics

- 8% of human genome, vertically inherited in the germ line
 - Proviral genes have insertions and deletions which compromise the virus's ability to replicate
 - HERVs are also under epigenetic silencing by methylation including by DNMT in epithelial cells. SWI/SWF which controls gene expression by modulation histone marks such as H3K9 germ line cells and becomes hypomethylated during carcinogenesis.
 - Some but not all HERV have open reading frames permitting translation
 - Retroviral self classified into 30 distinct families, 100 of which are HERV-K, which differentially affect people.
 - Individual differences in HERV-ome influence species evolution, individual traits, and susceptibility to disease.
 - HERV is somewhere between a normal gene and a virus, and *immunologic tolerance is imperfect*.
 - Retroviral promoters consisting of LTRs are capable of initiating viral transcription if they are not epigenetically silenced.

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HERV Basics, continued

- HERV's are implicated in glioma, breast, prostate, melanoma, colon, ovarian cancer, testes cancer, Hodgkin disease, renal cell carcinoma, urothelial cancer, neuroblastoma, CML, and sarcoma
 - HERV-K (HML2) is an envelope protein expressed in majority of women in US and China with breast cancer
 - HERV-K antibodies are present in the plasma of testicular cancer patients
 - HERV reactivation is heavily implicated in transplant-related cancers
 - HERV antibodies found in abundance in the sera of patients with autoimmune diseases, including, RA, SLE, MS, and ALS.
 - HERV expression can be triggered by other viral infections.

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HERV Basics, continued

- Retrovirus are ds, positive sense RNA viruses with reverse transcriptase (RT) which allows integration into host DNA mediated by integrase (IN) to create a provirus.
- HERVs are composed of *gag*, *pro*, *pol*, and *env* regions sandwiched between long terminal repeats (LTR's)
 - LTR's contain promoters, enhancers, and transactivation elements that regulate HERV transcription.
 - *gag* codes for individual proteins
 - *pol* codes for RT, IN, and RNase H
- HERV-K retain ORF's for all viral proteins
- HERV-K has 11 subgroups
- HERV-K/H/R/T have been detected in patients with lung and breast cancers.
- HERV expression can be induced by other virus (CMV, HBV, HIV, influenza A, herpes viruses – HHV8, EBV, HTLV-1)

Chen, et al. Oncogenesis, 2019, 8:6

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HERV Perpetrator

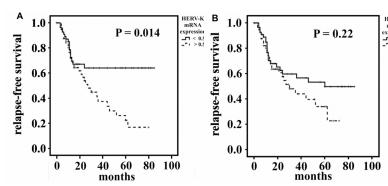
- HERV perpetrate oncogenesis*
 - HERV-K viral proteins (rec and np9) interact with the PLZF repression of the cMYC oncogene and and LNX repression of NOTCH (np9)
 - TZFP involved in AR repression. (Rec)
 - LTRs can activate the promoters of oncogenes (CSF1R)
- HERV envelope proteins promote immune escape**
 - (ii) Rec/Np9: c-MYC derepression and androgen receptor deregulation

*Bannert M, et al. Frontiers in Microbiology, 2018 9:178
** Beachboard, et al. Current Opinions in Microbiol, 2016,32, 113-9

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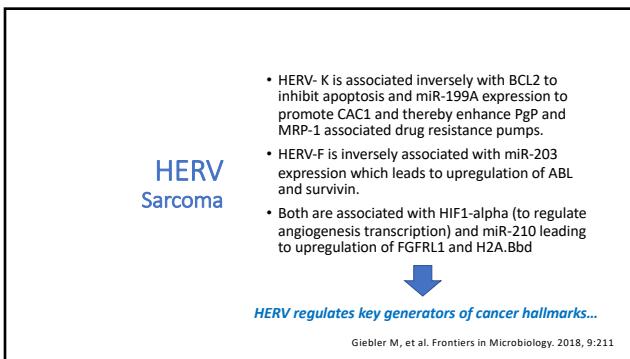
HERV Sarcoma

- In sarcoma, both HERV-K and HERV-F are inversely associated with relapse free survival.
- HR 1.78 for HERV-K and 2.0 when both were elevated.

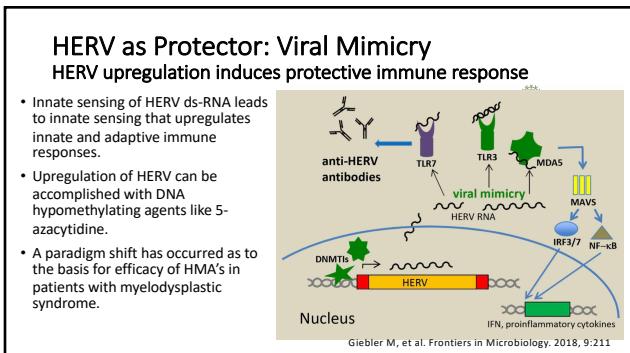


Giebler M, et al. Elevated HERV-K Expression in Soft Tissue Sarcoma Is Associated with Worsened Relapse-Free Survival. Frontiers in Microbiology. 2018, 9:211

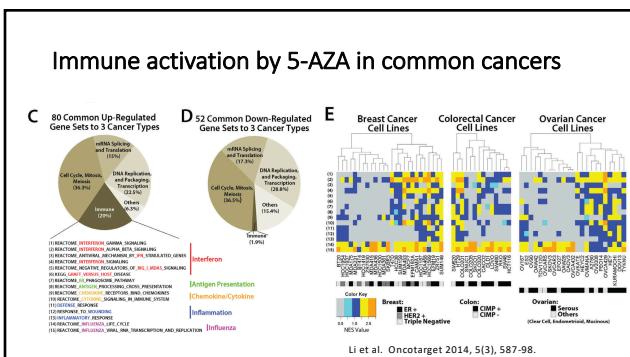
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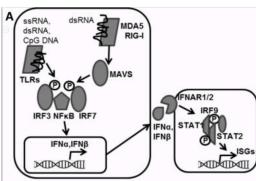
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5-AZA induces expression of multiple DNA hypermethylated endogenous HERVs

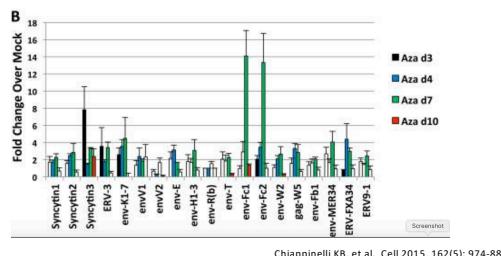
- The major mechanism underlying the Aza-triggered immune response is induction of a cytosolic double-stranded RNA (dsRNA) sensing pathway used by epithelial and other cell types as a viral defense mechanism that triggers a Type I interferon response
- Aza-induced viral defense genes and *IFNB1* are not DNA-methylated at promoter regions
- Aza activates Type I IFN- β mediated signaling through JAK/STAT



Chiappinelli KB, et al. Cell 2015, 162(5): 974-88

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Aza upregulates sense and antisense ERV transcripts

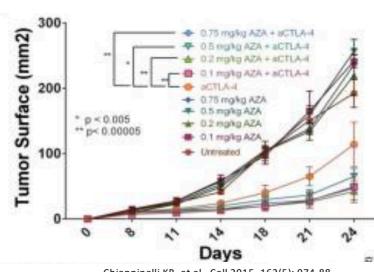


Chiappinelli KB, et al. Cell 2015, 162(5): 974-88

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Cold v. HOT HERV tumors

- Some cancers have low baseline HERV expression, and others 12.65-fold more.
- Tumors with greater expression have robust responses to CTLA-4i.
- Aza treatment potentiates immune checkpoint therapy in a mouse model of melanoma



Chiappinelli KB, et al. Cell 2015, 162(5): 974-88

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From Theory to Practice

- Viral RNA sensing genes and type 1 IFN response are indicative of HERV expression and appear to represent a biomarker of response to immunotherapy.

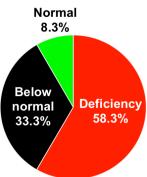
PROPOSAL

- Measure gene expression signature related to HERV expression, Type 1 IFN, and ds-RNA sensing.
- If absent, we create a rationale for hypomethylating therapy (5-AZA) to turn a HERV-cold tumor hot in combination with immunotherapy (anti-PD1/L1).

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Vitamin C increases viral mimicry induced by 5-aza

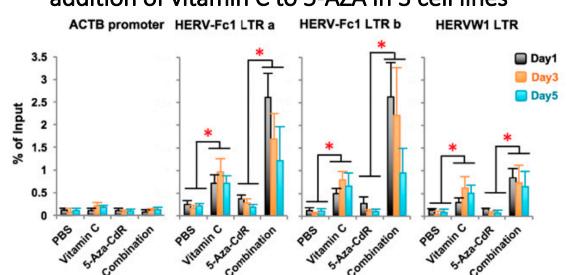
- TET enzymes convert 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) to deactivate the methylation mark.
- However, 5hmC depletion in a variety of human cancers suggests decreased TET activity in cancer cells
- Addition of vitamin C to ES cells promotes DNA demethylation by increasing TET activity
- A majority of patients with AML have below normal or deficient levels of vitamin C



Liu, et al. PNAS 2016, 113(37): 10238-10244.

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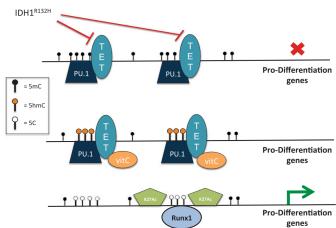
Upregulation of immune and HERV genes by addition of vitamin C to 5-AZA in 3 cell lines



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Vitamin C as epigenetic therapy for IDH-mutated cancer

- Normally the oncometabolite 2HG arising as a consequence of IDH1/2 mutations inhibits TET.
- Vitamin C overcomes TET inhibition and can force the expression of pro-differentiation genes.

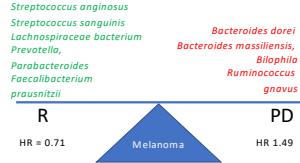


M Mingay, et al. Vitamin C-induced epigenomic remodelling in IDH1 mutant acute myeloid leukaemia. Leukemia (2018) 32, 11–20

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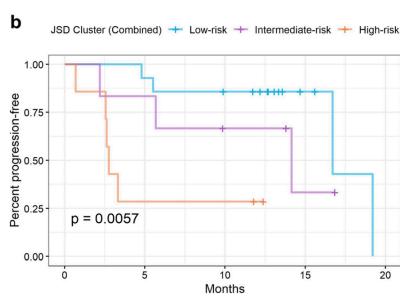
Microbiome

- Zitvogel and Gaewski discovered that the efficacy of immunotherapy depends on the presence of gram(+) bacteria in the gut microbiome, including *Bifidobacterium bifidus*, *Akermansia muciniphila*, and *Enterococcus hirae*.
 - The organisms educate the T cells to express chemokine receptors (CXCR3) which allows the immune cells to track into the tumor microenvironment.
- Clinical confirmation of the crucial nature of microbiome composition was reported in a study of checkpoint immunotherapy in metastatic melanoma patients treated at MD Anderson Cancer Center.



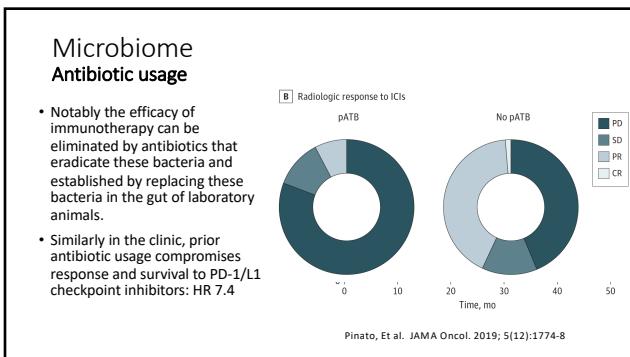
Peters B., et al. Journal of Clinical Oncology 2018 36:15_suppl, 9575-9575

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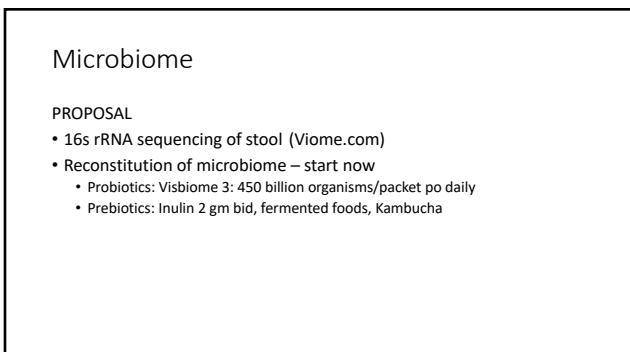


Peters B, et al. Genome Med, 2019, 11:61

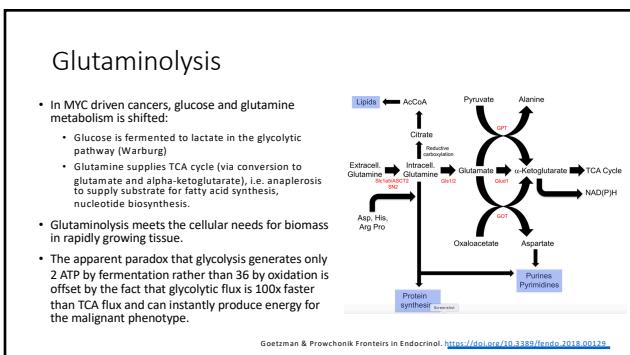
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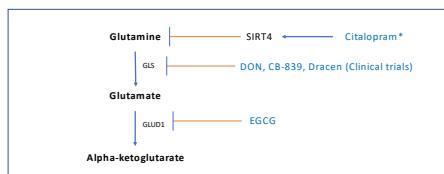


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Potential Therapies

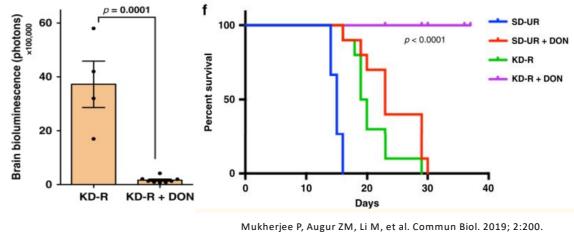


Should we try citalopram and EGCG?

*Buler M, et al. FASEB J. 30, 3942-3960 (2016)

70

Targeting Metabolism Glutaminolysis + ketogenic diet/calorie restriction



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Taurine

- Semi-essential amino acid, produced in small quantities in the body.
- Protective role in metabolism, vasculature, cardiac function, retina, lens, and brain
- Touted and used as an antiseizure medicine in large doses
- Vegans have lower taurine levels and are at risk of taurine deficiency
- Taurine supplements are protective against retinal disease, and tinnitus, lower catecholamine levels substantially, reduce blood pressure, reduce fasting blood glucose.

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Taurine and Cancer

- Low levels of taurine are consistently present in patients diagnosed with colorectal cancer, and may serve as a biomarker for that diagnosis
- Implicated in apoptotic thermostatic setting BCL2/BAX ratio and determining apoptotic fate – survival v. cell death – in lung, hepatoma,^{**} and breast cancer cell lines.^{***}
- Coadministration of taurine and cisplatin is synergistic in killing cervical cancer cells.^{****}

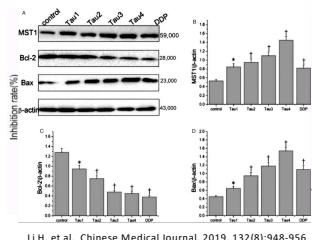
^{*}Tu S, et al. *Oncol Lett.* 2018;15(4):5473–5480.
^{**}Tu S., et al. (2015). Experimental and Therapeutic Medicine, 10, 193-200.
^{***}Zhang, X., et al. (2015). International Journal of Molecular Medicine, 35, 218-226.
^{****}Kim I, et al. *Adv Environ Biol.* 2013;776:189-98.

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Taurine effects on cervix cancer

- Inhibits proliferation and upregulates apoptosis in cervix cancer cells
- Taurine upregulating the expression of the proapoptotic proteins p73, p53, PUMA (p53 upregulated modulator of apoptosis), and caspase-3, and promoted the phosphorylation of YAP (Yes-associated protein).

PROPOSAL: measure taurine and consider supplementation if levels are low



Li H, et al. *Chinese Medical Journal.* 2019, 132(8):948-956

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Summary of Recommendations

Diagnosis

- Review pathology for T cell infiltration
- Methylome
- Transcriptome for Gene expression
 - Subtyping tumor (SHh, MYC, TYR)
 - Assessment for HERV expression, Type 1 IFN response, ds-RNA sensing
 - Immunomic Panel (Tempus, Alacris)
- Measure taurine and ascorbate levels
- Microbiome evaluation: 16s rRNA stool sequencing

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Summary of Recommendations

Therapy

- Resume Tazemetostat
- Commence HDACi: valproic acid ER
- Continue PD-1/L1i: nivolumab (Opdivo™)
- If HERV, Type 1 IN response, or T cell infiltrate in original tumor is missing: 5-Azacytidine 75 mg/m² x 7 days combined with vitamin C
- Microbiome reconstitution
- Revise immunotherapy strategy following availability of immunomic profile
- Consider taurine supplementation: 1-2 gm BID
- Re-Consider targeted therapy options once pathway upregulation has been identified
- If MYC is upregulated:
 - Glutaminolysis approach (CB-839 clinical trial) combined with ketogenic diet, or
 - EGCG 1,000 mg q4h x 3-4x per day with de-ionized water, Fish oil 1,000 mg, vitamin C 200 mg
 - Citalopram 10-40 mg to upregulate SIRT4 to diminish glutaminolysis
