Report from 2015 American Association for Cancer Research Annual Meeting, Philadelphia, PA, USA, April 18-22, 2015 – Dominika Nowis

The AACR Annual Meetings highlight the latest, most exciting discoveries in every area of cancer research and provide a unique opportunity for over 15,000 investigators from all over the world to meet, interact, and share their insights. 2015 year's meeting theme – "Bringing Cancer Discoveries to Patients" – underscores the vital and inextricable link between discovery and treatment, and it reinforces the fact that research underpins all the progress we are making in the field toward cancer cures. This motto perfectly fits the principles and goals of the BASTION project.

Dr. Dominika Nowis has an opportunity to present there the results of one of her latest research projects during the poster session on Experimental and Molecular Therapeutics - Drug Discovery on Wednesday, April 22, 2015.

Poster title: **SK053, a small molecule inhibitor of enzymes involved in allosteric disulfide bonds formation, shows potent anti-leukemic effects and induces differentiation of human AML cells**

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2Laboratory of Experimental Medicine, Center of New Technologies, University of Warsaw, Warsaw, Poland
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Abstract: Although differentiation-inducing agents have significantly improved the management of acute promyelocytic leukemia, no significant progress has been made in the treatment of other acute myeloid leukemias (AML). Numerous proteins involved in tumor development have so-called allosteric disulfide bonds amenable to

modifications affecting protein structure and function. We have developed SK053, a small molecule and mechanism-selective inhibitor of enzymes involved in allosteric disulfide bonds formation such as thioredoxin, thioredoxin reductase and protein disulfide isomerase (PDI). The aim of our studies was to evaluate anti-leukemic activity of SK053 in human AML cells. To validate if SK053 targets PDI, a binding assay and an insulin turbidimetric activity assay were used. Cytostatic/cytotoxic effects in HL60, NB4, KG-1 and MOLM14 cells as well as in primary AML cells were assessed with trypan blue exclusion. Differentiation of AML cells was studied with May-Grünwald-Giemsa staining, nitro blue tetrazolium reduction assay and flow cytometry analysis of CD11b, CD14 and CD15 levels and by RNA sequencing, qRT-PCR and western blotting (WB). We observed covalent binding of SK053 to PDI and inhibition of its enzymatic activity with IC_{50} of 10 μM. Since PDI blocks translation of CCAAT enhancer binding protein alpha (CEBPA), a transcription factor crucial for neutrophils maturation, we evaluated the potential of SK053 to induce differentiation and cytostatic/cytotoxic effects in human AML cells. SK053 exerts significant cytostatic/cytotoxic activity in human AML cells (HL60, NB4, KG-1 and MOLM14), and induces differentiation of AML blasts into more mature myeloid cells. Incubation of AML cells with SK053 induced expression of CEBPA and hexokinase 3 mRNA in quantitative RT-PCR and increased amount of CEBPA protein in nuclear fraction measured in WB. Finally, SK053 induces differentiation of primary leukemic cells freshly isolated from AML patients. RNA-seq analysis revealed that incubation of HL60 cells with SK053 down-regulates mRNA for MYC and ID1 oncogenes as well as for histone proteins. Expression of other genes of mature myeloid lineage such as adhesion molecules (collagen type XV, fibronectin I, MAC-1), hydrolytic enzymes (carboxypeptidase, proteinase 3, CA12 anhydrase, ADAM19 metalloprotease), proteoglycan 2 (core of eosinophilic granules) and PGLYRP3 (peptidoglycan recognition protein 3) was significantly up-regulated. The GeneOntology analysis done with the RNAseq results revealed enrichment of gene transcripts regulating myeloid cells differentiation. SK053 exerts potent anti-leukemic activity and induces differentiation of numerous types of human AML cells. Targeting allosteric disulfide bonds with small molecule inhibitors presents a promising therapeutic strategy in AML.

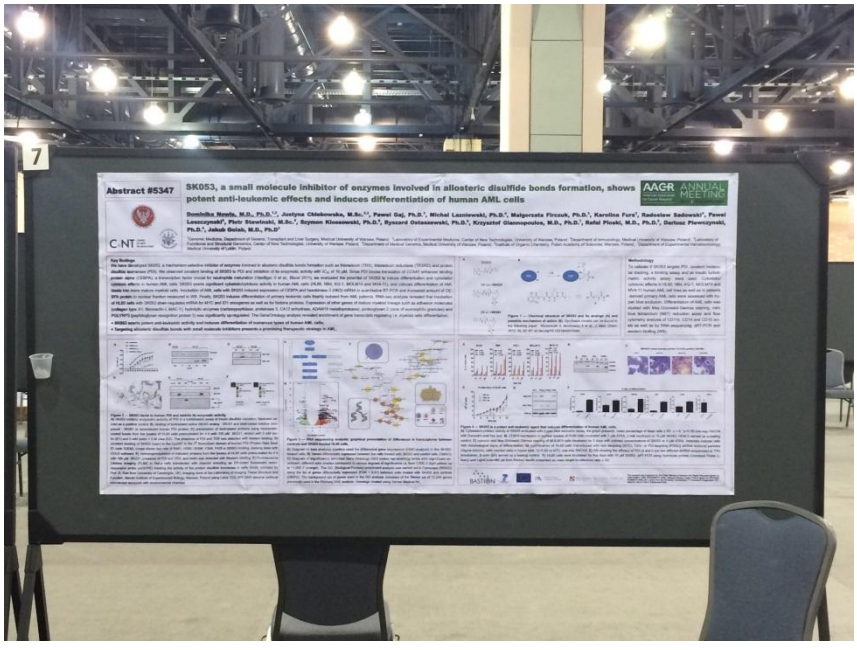

Fig. 1 – The poster presented by Dr. Nowis during 2015 AACR Annual Meeting

This presentation drew attention of several scientists. Dr. Nowis answered numerous questions dealing with the design and results of her study and got several poster pdf requests.

Moreover, on April 27, 2015 at the weekly meeting of the Department of Immunology, MUW, Dr. Nowis gave a talk highlighting the most important information she learnt during the meeting.

Below - Slides from Dr. Nowis' presentation on 2015 AACR Annual Meeting highlights given at the Department of Immunology, MUW:

Highlights from 2015 AACR annual meeting

Dominika Nowis
April 27, 2015

Meeting on tumor immunology and immunotherapy

- Glenn Dranoff
- Ira Mellman
- James P. Allison
- Jedd D. Wolchok
- Ton N. Schumacher
- Robert Schreiber
- David N. Munn
- Antoni Ribas
- F. Stephen Hodi
- Drew Pardoll
- Guido Kroemmer
- Nicholas Restifo
- ...

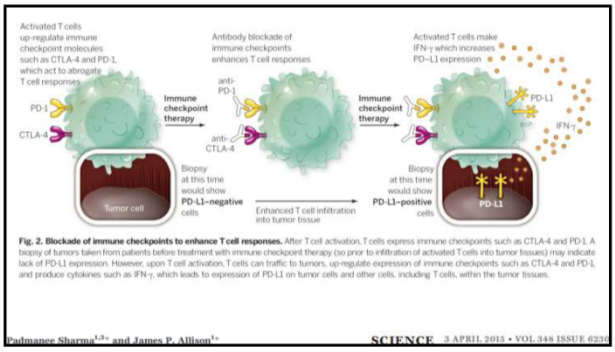

Fig. 2. Blockade of immune checkpoints to enhance T cell responses. After T cell activation, T cells express immune checkpoints such as CTLA-4 and PD-1. A biopsy of tumors taken from patients before treatment with immune checkpoint therapy (so prior to infiltration of activated T cells into tumor tissues) may indicate lack of PD-L1 expression. However, upon T cell activation, T cells can traffic to tumors, up-regulate expression of immune checkpoints such as CTLA-4 and PD-1, and produce cytokines such as IFN-γ, which leads to expression of PD-L1 on tumor cells and other cells, including T cells, within the tumor tissues.

Padmanee Sharma[1,2]* and James P. Allison[1]*
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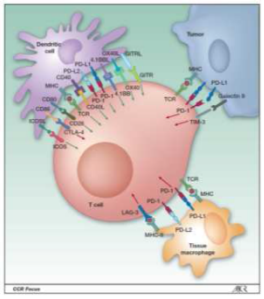


Anti-CTLA4	ipilimumab	melanoma	2011
Anti-PD1	pembrolizumab	melanoma	2014
Anti-PD1	nivolumab	melanoma	2014
		lung cancer	2015

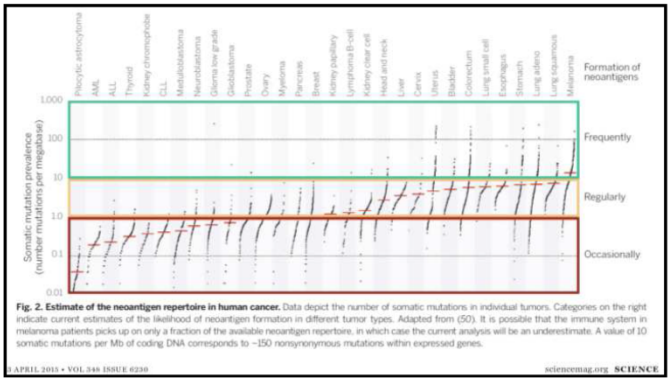
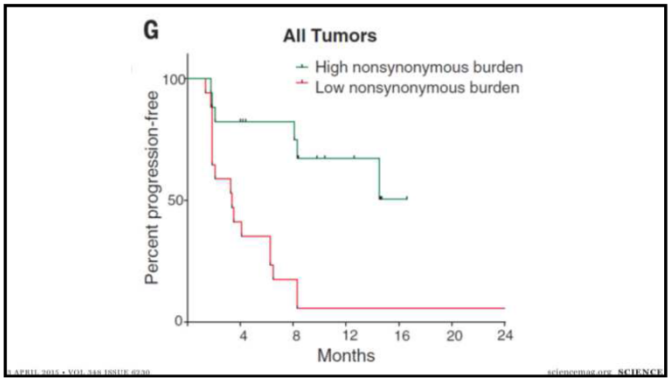


Fig. 2. Estimate of the neoantigen repertoire in human cancer. Data depict the number of somatic mutations in individual tumors. Categories on the right indicate current estimates of the likelihood of neoantigen formation in different tumor types. Adapted from (50). It is possible that the immune system in melanoma patients picks up on only a fraction of the available neoantigen repertoire, in which case the current analysis will be an underestimate. A value of 10 somatic mutations per Mb of coding DNA corresponds to ~150 nonsynonymous mutations within expressed genes.



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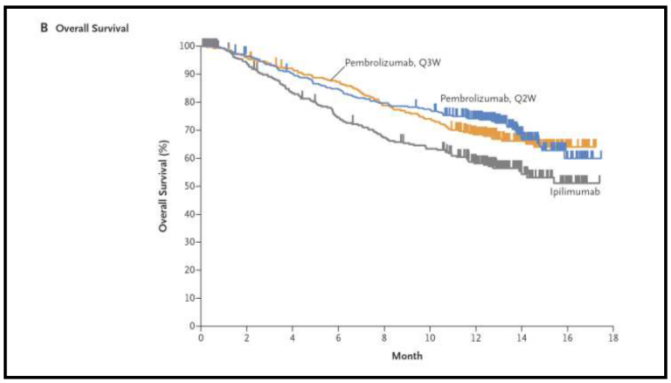
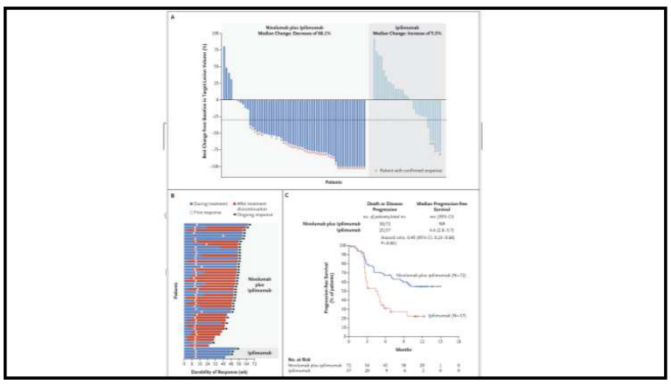
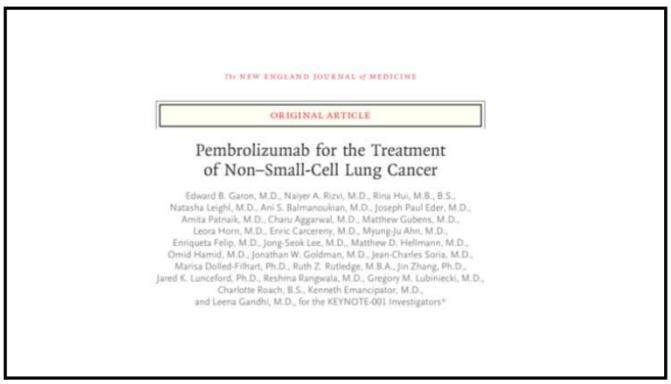
Is recognition of neoantigens important for the efficacy of immunotherapy?

- Higher mutational load – increased intratumoral expression of genes reflecting T cells and NK cells activity
- More DNA damage – better induction of anti-tumor immune response
- More mutations – better response to checkpoint blockade in NSCLC

Ton N. Schumacher







The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer

Edward B. Garon, M.D., Naiyer A. Rizvi, M.D., Rina Hui, M.B., B.S., Natasha Leighl, M.D., Ani S. Balmanoukian, M.D., Joseph Paul Eder, M.D., Amita Patnaik, M.D., Charu Aggarwal, M.D., Matthew Gubens, M.D., Leora Horn, M.D., Enric Carcereny, M.D., Myung-Ju Ahn, M.D., Enriqueta Felip, M.D., Jong-Seok Lee, M.D., Matthew D. Hellmann, M.D., Omid Hamid, M.D., Jonathan W. Goldman, M.D., Jean-Charles Soria, M.D., Marisa Dolled-Filhart, Ph.D., Ruth Z. Rutledge, M.B.A., Jin Zhang, Ph.D., Jared K. Lunceford, Ph.D., Reshma Rangwala, M.D., Gregory M. Lubiniecki, M.D., Charlotte Roach, B.S., Kenneth Emancipator, M.D., and Leena Gandhi, M.D., for the KEYNOTE-001 Investigators*

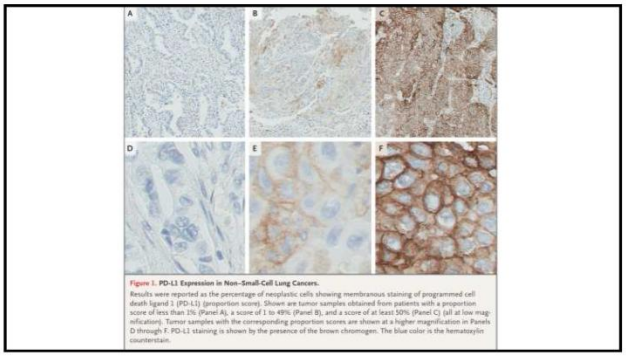
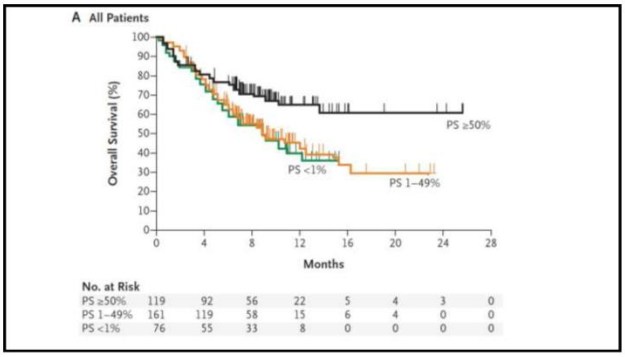


Figure 1. PD-L1 Expression in Non–Small-Cell Lung Cancers.
Results were reported as the percentage of neoplastic cells showing membranous staining of programmed cell death ligand 1 (PD-L1) (proportion score). Shown are tumor samples obtained from patients with a proportion score of less than 1% (Panel A), a score of 1 to 49% (Panel B), and a score of at least 50% (Panel C) (all at low magnification). Tumor samples with the corresponding proportion scores are shown at a higher magnification in Panels D through F. PD-L1 staining is shown by the presence of the brown chromogen. The blue color is the hematoxylin counterstain.



Natural killer (NK) cells

- Memory NK cells are induced by cytokines, haptens, viruses (HIV, HBV, CMV)
- Cytokine (IL-12, IL-15, IL-18) -induced memory-like NK cells (CIMS) – have superior anti-tumor activity than regular NKs
- Phase I study of CIMLs in AML
- IL-15R-IgGFc (ALT-803) + rituximab – phase I/II studies

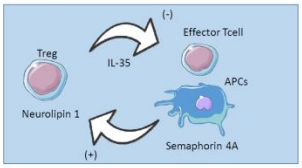
Todd Fehniger

Targeting Tregs in tumor microenvironment

1) Targeting Tregs migration
- CXCR4 modulation

2) Targeting the suppressive mechnisms of Tregs
- anti-IL35 MAbs

3) Targeting Tregs stability survival
- anti-neurolipin1 MAbs
- anti-semaphorin 4A MAbs



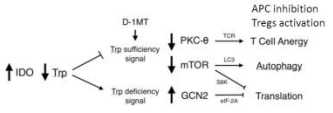
Dario Vignali

IDO (Indoleamine 2,3-dioxygenase)

Biomarkers of IDO caveats:

1. No standarized antibodies for IHC
2. Absence of IDO in the initial tumor biopsy does not mean IDO is not there (It may be expressed at later tumor stage or in the tumor-draining lymph nodes)
3. Serum kynurenine levels are not a sensitive or specific biomarker of IDO in the tumor

IDO inhibitors (INDOXIMOD, NLG919) are in the clinical trials in combination with chemo- and immunotherapy



Tryptophan metabolites are recognized by AHR (aryl hydrocarbon receptors) that results in Tregs activation and induction of tolerogenic DCs

David H. Munn

Trageting MDSCs

- Inhibition of CSF1R signaling
- Inhibition of IL10R
- Inhibition of CXCR4
- Inhibition of IDO
- Inhibition of FasL
- Inhibition of VEGF/VEGFR
- Inhibition of CCR2/GM-CSF
- TNFalpha delivery
- Targeting cEBPbeta
- Targeting GATA6
- Targeting TRIB1

transcription factors crucial for MDSCs

- Targeting miR-142-3p

Single targeting of MDSCs will not be sufficent and should rather be combined with other methods of immunotherapy

Vincenzo Bronte

Use of CRISPR/Cas9 technology in vivo:

- Technology announced in 2012
- So far over 1300 CRISPR/Cas9 papers have been published
- Germline Cas9 expression in mice (F. Zhang, Cell 2014)
- Evaluation of the role of GOI in cancer development (gain-of-function mutations – e.g. beta-catenin; loss-of-function mutations – e.g. PTEN)
- CRISPR/Cas9 + CRE – phenotype observed in mice in 10 weeks post induction

Tyler Jacks

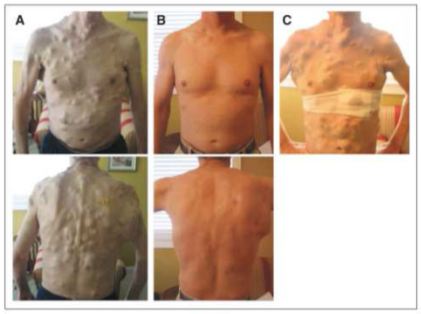
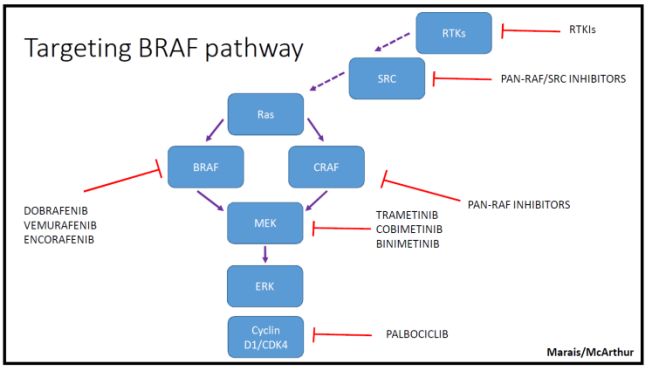


Fig 2. A 38-year-old man with *BRAF*-mutant melanoma and miliary, subcutaneous metastatic deposits. Photographs were taken (A) before initiation of PLX4032, (B) after 15 weeks of therapy with PLX4032, and (C) after relapse, after 23 weeks of therapy.

Targeting BRAF pathway

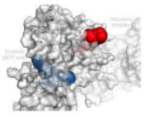


Marais/McArthur

Dual targeting of BCR-ABL with ABL001 and nilotinib in CML

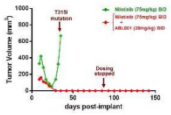
Tasigna® in Combination with an Allosteric BCR-ABL Inhibitor Could Advance Treatment of CML

Simultaneous binding of two inhibitors to BCR-ABL



- ABL001 is a highly potent and selective allosteric inhibitor of BCR-ABL
- ABL001 binds to BCR-ABL in combination with nilotinib or imatinib without cross-competition

In preclinical model, ABL001/nilotinib combination appears to prevent emergence of resistance[1]

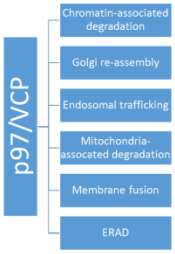


- Combinations of the two inhibitors prevent emergence of resistance in a blast crisis CML xenograft model
- Phase I study has been initiated

NOVARTIS

William Sellers

Targeting AAA (ATPases associated with diverse cellular activities) p97/VCP



Inhibitor(s) show efficacy in myeloma:

- Cell lines (>300)
- Bortezomib-refractory myeloma patients cells
- MM1S-luc xenograft model in mice
- Vk*Myc mouse myeloma model
- Phase 1 trials in myeloma and solid tumors

N N N

C>A
C>G
C>T
T>A
T>C
T>G

6 variants

x 4 for left

AC>A AC>G AC>T AT>A AT>C AT>G
CC>A CC>G CC>T CT>A CT>C CT>G
TC>A TC>G TC>T TT>A TT>C TT>G
GC>A GC>G GC>T GT>A GT>C GT>G

24 variants

x 4 for right = 96 signatures

Michael Stratton

Validated mutational signatures found in human cancer

Signature	Causing mechanism
2, 13	APOBECs activation
7	Tobacco
24	Aflatoxin
6, 15, 20, 26	Defects in mismatch repair
3	Mutated BRCA1/BRCA2

nature

LB Alexandrov *et al. Nature* 000, 1-7 (2013) doi:10.1038/nature12477

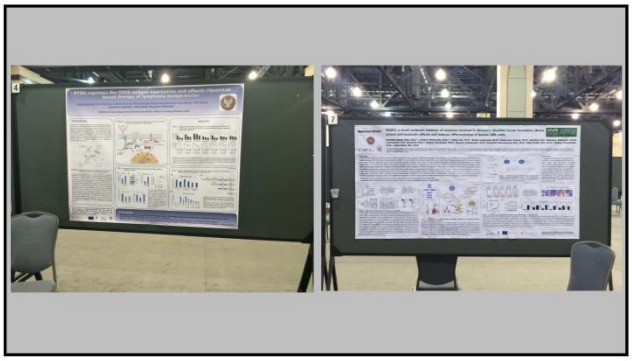
Driving mutations in non-coding transcriptome

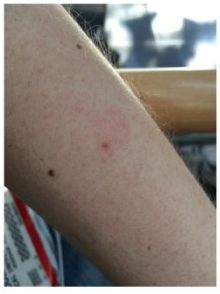
- 3,000 miRNAs
- 38,000 lncRNAs
- 20,000 pseudogenes
- 100,000 circular RNAs

Other (chosen) topics:

- Mechanisms of synthetic lethality (Alan Ashworth)
- Combination of targeted therapy and immunotherapy:

BRAF inh + anti-CTLA4 (Jedd Wolchok)
BRAF inh + anti-PD1 (Antoni Ribas)
BRAF inh + TILs (Nicholas Restifo)
Time to go beyond BRAF inhibitors and melanoma....
TILs + AKT1/PKB inhibitors (AKT drives T cel exhaustion) (N. Restifo)
PPAR gamma agonists + anti-tumor vaccines (GVAX) (Glenn Dranoff)





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