As part of our ongoing coverage of the 2017 American Association for Cancer Research (AACR) meeting, we attended multiple oral presentations on April 3rd and our notes are below. Affected Companies include Jounce Therapeutics (NasdaqGS: JNCE), Juno Therapeutics (NasdaqGS: JUNO), Kite Therapeutics (NasdaqGS: KITE), Cellectis (NasdaqGM: CLLS), Agenus (NasdaqCM: AGEN), Advaxis (NasdaqCM: ADXS), Amgen (NasdaqGS: AMGN), Salarius Pharmaceuticals (private), Neon Therapeutics (private), Novartis (NYSE: NVS), Bristol-Myers Squibb (NYSE: BMY), and Merck (NYSE: MRK).

Session Notes: The Immune System and Cancer

James P. Allison: Immune checkpoint blockade in cancer therapy: Insights, opportunities, and prospects for cures

Companies Affected: Bristol-Myers Squibb, Merck, Jounce, Agenus

- First checkpoint approval was Yervoy in 2011. Took 3 years for first PD-1 approval. 5 approvals in 2015. 5 approvals in 2016 with checkpoints. 3 in 2017 already.
- CTLA-4 blockade enhances tumor specific immune response.
- Immune checkpoint blockade doesn't target tumor cells, doesn't involve vaccines or cytokines to turn on immune responses. Works by blocking inhibitory pathways to unleash anti cancer immune responses.
- CTLA-4 accumulates in cell over time, and reaches level where it outcompetes CD28 for binding of B7. Affinity 3 times higher than CD28.
- Immune response doesn't start until sufficient tumor cell death so that dendritic cells can present antigens to T cell.
- CTLA-4 knockout kills mice. Need CTLA-4 to some degree.
- CTLA-4 blockade turns T cells off before tumor eliminated.
- Because process initiated by tumor cell death, use with chemo is rational.
- Made difference between death in mice and curing tumor with CTLA-4. Expected it to slow tumors down, but surprised that so potent.
- Medarex made CTLA-4 fully human antibody. About 70,000 treated to date. Objective responses in many tumors like melanoma, prostate, kidney, bladder, ovarian, lung, etc.
- AEs include colitis hepatitis, hypophysitis, Serious but generally management.
- 3 year OS rate of 21%.
- Median OS of 9.5 months.
- Yervoy moved median survival over by 3 months vs. gp100. Real benefit is flattened survival curve. Long-term durable responses.
- About 21% of patients treated with Yervoy alive at 10 years.
- PD-L1 induced resistance mechanism 9can come from IFN-y), whereas CTLA-4 is more standardized.
- Prostate cancer talk tomorrow on how to get patients to respond to PD-1.
- Combination of Yervoy and PD-1 very effective.
- 60% survival at years with combo. Can treat half of metastatic melanoma patients.
- CTLA-4 and PD-1 are very different.
- CTLA-4 – hard wired, targets CD28 pathway, works during priming, expands clonal diversity, primarily effects CD4 T cells, can move T cells into tumor, responses often slow, disease recurrence after response.
- PD-1 – induced resistance targets TCR pathway, works on exhausted T cells, does not expand clonal diversity, primarily effects CD8 T cells, does not move T cells into tumors, responses usually rapid, disease recurrence after response significant.
- With CTLA-4 get positive ICOS Th1 like cells. Main change with PD-1 is TIM3 increases showing exhausted cells. Get this same thing in different tumor models.
- As you might expect, as you have KLRG1+, with increased tumor growth, get a lot of T regs. ICOS+ Th1=like associated with smaller tumor size.
- Specific CD8 populations correlate with outcome.
- The therapeutic mechanism of anti CTLA-4 and anti PD-1 are distinct. May explain the synergies.
- Specific CD4 and CDD8 T cell subtypes contribute to the therapeutic effect in both treatments.
- ICOS – member of CD28/CTLA4 superfamily. Usually associated with Tfh or Treg.
- Pam sharma showed ICOS + Th1 CD4 associated with Yervoy treatment.
- Combination of IVAX and anti-CTLA-4 enhances IFN-y an TNF-a production by CD4 and FOXP3 cells.

**Laurence Zitvogel: Microbiome and cancer therapies**

**Company Affected: N/A**

- 17% of human malignancies are void of T cell infiltration. Could be that T cells not primed in first place, or several suppressors to inhibit their infiltration.
- CD*+ T cell density is crucial, associated with high mutational load, and high PD-L1 expression.
- Microbiome is controlling cancer immune set point.
- Intestinal barrier has large body of immune cells. Have trillions of microbes that exert a myriad of functions.
- Involved in chronic immune diseases.
- Gut microbiota impacts on therapy induced antitumor immune-surveillance.
- Could a minimalist microbiome normalize the cancer immune set point?
- Antibiotics reduce survival of advanced cancer patients treated with PD1 inhibitors. Compromised OS and PFS.
- Antibiotics enter guy, did metagenomics analyses.
- Contrasted responders and non-responders.
- Top bacterium is akermansia muciniphila – abundance of this in responders. Famous
- Did fecal microbial transplant in mice: experimental setting.
- Feces composition influences the antitumor efficacy of anti-PD1 Abs in avatar mice.
- Dysbiosis accounts for the lack of response to aPD1 Ab in avatar mice.
- Anticancer probiotics – looked at A. muc and E. hirae.
- The combination of E. hirae + A. muciniphila is very efficient in ATB-induced dysbiosis.
- FMT-induced dysbiosis: restoration of aPD1 Abs efficacy with anticancer probiotics.
- Anticancer probiotics activate mesenteric T cells found in the tumor beds.
- Cold tumor could become hot with proper anti-cancer probiotics.

**Crystal L. Mackall: Genetically engineered T cells for cancer**

**Companies Affected: Juno, Kite, Cellectis, Novartis**

- Response to checkpoint has varied widely, many factors impacting response. Big one is mutational burden.
- Many things can be added to checkpoints called immune response amplifiers.
- If non-immunogenic cancer, can do immune response initiators: synthetic immunotherapies. mAb, bispecific, CAR T.
- First CAR could be approved in 2017.
- CD-19 CAR therapy has high CR rate in B cell malignancies.
  - 70-93% CR in R/R B-ALL.
  - 40-55% CR in chemo-resistant lymphoma.
  - Similar response rates across trials employing varying vectors.
  - Response correlated with CAR-T 100 fold expansion of CD19 CAR-T cells with 7-14 days following infusion.
- Lymphodepleting preparative regimen is essential for efficacy, with cyclophosphamide/fludarabine being the current gold standard.
  - Previous allo HSCT does not impact response or toxicity.
  - Doses of CD19-CAR T cells needed for efficacy <<< for other adoptive T cell therapies.
  - Some ideas to diminish toxicity in high disease burden is to give a lower dose of CAR-T.
  - Clearance of high burden CNS leukemia by CAR T cells.
- CRS biggest toxicity with CAR –T. occurs 7-14 days after infusion. Many inflammatory cytokines. Anti-IL-6 is very effective to mitigate toxicity.
- Grade based treatment algorithms has improved outcomes and reduced SAEs.
- High grade CRS occurs in about 25% of patients.
- Other SAE is reversible neurotoxicity. Occurs in about 50% of patients, and more common in CRS patients.
- Lethal neurotoxicity is less than 1%. Unknown exactly why it occurs.
- Resistance – antigen loss is most common form of resistance. 26% of responders relapsed with CD19 negative B-ALL with median follow up of 10 months.
- More so isoform switch instead of antigen loss.
- One way to deal with antigen loss is to go after other antigen. CD22 is target being developed.
- CD22 similar to CD19 in behavior and response.
- Antigen loss can occur in CD22 as well.
- Down regulation of CD22 is sufficient for leukemic escape.
- Options for simultaneous targeting of two antigens by CAR-T cells.
- For CARs, need to think about how to target multiple antigens at same time. Can do by co-expressing or co delivering cells. Multi-gene integration with CRSPR CAS-9.
- Bivalent-bispecific receptor being developed at NCI.
- 73% CR with CD22 with equivalent dose to CD19 CAR.
- CD19-CAR is not unique.
- Antigen loss escape is likely to be magnified in solid tumors.
- Optimizing engineering of multi-antigen targeted CAR_T cells will be important for expanding progress with CAR-T cell therapeutics.
- 4-1BB costimulation protective against T cell exhaustion increases cytokine production and anti tumor activity.
- Biological basis for this phenomenon is unknown.
- CD39 good marker for exhausted cells.
- Working to stabilize CAR expression. Can be regulated up to 30 fold.
- Destabilizing GD2.28.z-CAR T cells do not exhaust due to an absence of tonic signaling, but transient CAR upregulation enables antigen driven CAR activation.
- 2017 CARs – monospecific, constitutive expression unregulatable, engineering not optimized, cells are often short lived, genes inserted randomly into genome and stochastically into cell mixtures, do not leverage power of gene editing.
The next future of CARs – bi-specific, tri-specific or quad-specific receptors, remote controlled, logic gated, and or not gates, precision transgene, integration via CRISPR/Cas9 switch receptors to protect against immunosuppression, engineered to be exhaustion resistant, intelligent cells – able to integrate signaling and respond accordingly.

Levi A. Garraway: Cell states linked to immunotherapy response and resistance

Company Affected: Advaxis, Amgen, Neon

- Many challenges still in immunotherapy – majority of patients do not respond, determinants of response not well understood, approaches to rational combinations are poorly defined.
- Guiding principles: response to anticancer therapy – tumor dependence
  - Oncogene dependence – reliance of tumor cells on onco protein or downstream signaling pathway.
  - Lineage dependence – CDK4/6 inhibition form of this.
  - Synthetic lethal dependence- great example of this is PARP inhibition in BRCA or HRD cancers. Future could be PRMT5 inhibition (MTAP-depleted cancers).
- Immunologic dependence will be linked to cellular interaction in the tumor ecosystem and tumor cell autonomous effects are secondary.
- PD-L1 expression points to ecosystem effect instead of tumor cell autonomous effect.
- Mutational load and neoantigen load and cytolytic activity correlate with response.
- T cell lineages and functional states are readily discernible by single-cell analysis – could better predict which ecosystems will respond to immunotherapies.
- T cell receptor clusters suggests clonal expansion in tumor tissue.
- Functional states of clonally expanded T cell populations may influence immunotherapy effect.
- Targeted therapy – cell autonomous, mechanistic clarity, distinctions across tumor types.
- Immunologic dependence – cellular ecosystem, mechanistic uncertainty, tumor-type differences not yet well-defined.
- Resistance to targeted anticancer therapy. – Multi-factorial, heterogeneous, usually under-sampled in the clinic.
- Guiding principles: resistance to anticancer therapy – convergences.
- Convergence – involves downstream effectors, oncogenic output, alternative cell states.
- Neoantigen load predictive of survival.
- HLA mutations – often in peptide binding and TCR interaction.
- Greater infiltration of lymphocytes in tumor tissues, more likely to have HLA and antigen processing machinery mutation.
- Antigen processing is a convergence in immunotherapy setting.
- Immunotherapy-resistant T cells exhibit a higher exhaustion score.
- Tumor convergence – downstream effectors, oncogenic output, alternative cell states.
- Immunologic convergence – defective neo(antigen) processing, effector t cell dysfunction, alternative malignant and T cell states.
- Principles gleaned from targeted therapy may inform immunotherapy response and resistance.
- Immunologic dependencies seem fundamentally distinct from tumor dependencies.
- Convergene onto specific malignant cell and t cell aberrancies may cause immunotherapy resistance.

Session Notes: Epigenetic Therapy: What Is It, Where Are We, and What Does the Future Hold?

Jean-Pierre J. Issa: Targeting aberrant gene silencing in cancer
Company Affected: N/A

- What they have learned? Unlike cytotoxic therapy where tumor burden goes down very quickly, in hypomethylation and epigenetic therapy tumor burden is initially stable and then there is a reduction, sometimes after 4 mos. or even after 9 mos and it is not known why. He suggested maybe activation immune response.

- Biomarkers:
  - Mutation is TET, P53, DMNT3, or Ras status. Methylation. Gene expression signatures.
  - However, data so far does not provide a consensus.

- What is epigenetic therapy? Is it a: Differentiation response, apoptosis response, immune response. Therefore, the biomarkers are likely changing depending on the effects, and they are not analyzed as such so they have yet to find a strong biomarker.

- Next generation of trials have focused on histone modifiers.
  - HDAC inhibitors have shown to be active in certain classes of lymphoma but disappointing in other situations.
  - RCT with decitabine or azacitidine in combination with HDACS have largely failed.
  - So now there has been a focus on targeting other modifiers: K27me, Polycomb, K9me, K4me. But are these all the same, because they target K9 acetylation or are they different?
  - Genomic analysis found that DNMTi results in very specific gene regulation across tumor types, while in HDAC gene expression was extremely varied, not a clear correlation.
  - The drugs targeting the other marks namely, EZH2 and G9a inhibitors. These have demonstrated relatively mild effects on gene expression. 50-200 genes are upregulated and very little gene down regulation. Showed very good specificity.
    - Example, EZH2i mainly effects polycomb marked genes.

- The drugs effect a subset of about 8% of the genome, while HDAC inhibitors probably effect about 30% of the genome. As consequence, HDAC inhibitors have wide variability with respect to its effects in various indications, which complicates its clinical use.

- EZH2 and DNMT inhibitor combos were the best at activating tumor suppressor genes.

- G9A and DNMT inhibitor combination was better at inducing an immune response induction.

- LSD and DMNT combination were the best at inducing differentiation.

- Combination of these drug may lead to more precise effects

- He introduced the concept of precision epigenetic therapy after an epigenome analysis in order to target: tumor suppressors, differentiation, gene activation or inactivation, or immune activation.

- Currently wide variety clinical trials for epigenetic therapies that are either broad acting general programmers or more targeted; so-called precision epigenetic therapies. Some examples are: EZH2 inhibitors, IDH1 inhibitors, which are in various states of clinical development. However, he suggested that there is whole universe of epigenetic factor that may be missed by focusing on Histones and DNA methylation.

- Gave the example of a class of drugs call cardiac glycosides that are as effective as azacitidine. This class of drugs are new and have not been used in cancer and are now entering the clinical trials.

- New directions: drugs that change phenotypes and combinations of epigenetic therapy with other modalities (chemo or immunotherapy).

**Question**

- Q. Efficacy in heme cancers vs epithelial cancers? A. Unclear why heme cancers are more sensitive. They do see some response in lung. Heme cancers have more epigenetic mutations.

**Sunil Sharma:** Lysine specific demethylase-1 (LSD-1) inhibitors for treatment of cancer

Company Affected: Salarius Pharmaceuticals
Histone methylation is on the ways DNA packed. Lysine and arginine are marked through methylation and relay information.

- Methyltransferases: EZH1, 2 and DOTL.
- LSD1 is a demethylase. It can function as a corepressor or a coactivator. Acts K4 and K9 mark.
- LSD1 is amine oxides can demethylate of H3k9 mono and di methylation.
- Acts in different repression complexes such as NurD and Cores.
- LSD1 as repressor: part of the nuru complex, turning off enhancers during stem cell differentiation. Activator: shown to be activator of androgen receptor.
- LINC RNAs shown to coordinate demethylation by binding to LSD1 and other oncogenic factors to activate downstream cancer targets.
- LSD1 knockdown correlates with loss of leukemic stem cell potential of AML. Suggested it be used as putative therapy to differentiate leukemic cells.
- Ewing sarcoma and LSD1. 1000 adolescents are diagnosed per/year. Relapse is high.
- No effective treatment. IGF antibodies and PARPi are currently in development.
- LSD1 target EWS/FLI
- EWS/FLI interacts with Nurd complex, which contains LSD1. Presented an experiment showing dependence on LSD1 via chemical inhibition.
- LD1i preclinical in xenografts in mouse. In treated mice you see a slowdown in tumor growth, then a complete response.
- Showed LSD1 synergism with docetaxel in a xenograft model of prostate cancer.
- Carbo and LSD1 in a BC PDX showed efficacy.
- Types of LSD1 inhibitors: irreversible and reversible.
- Irreversible LSD1 inhibitors bind covalently. Likely to have adverse effects.
- Phase1 OR-1001. Were able to induce BM blast differentiation.
- Incyte has irreversible inhibitor in Phase I. GSK is developing one for AML.
- Salarius is planning Phase I in Ewing sarcoma, prostate cancer, TNBC in combination with carbo.

**Question**

- Q. Is the EWS-Fli the cleanest background to test this therapy. A. That indication is clean no secondary mutations…Other cancers may not be as straight forward. Probably other players involved …excited about LINC RNAs.

**Shelley L. Berger: Genomic and epigenomic mechanisms utilized by mutant p53**

**Company Affected: N/A**

- P53 mutated in over 50% human cancers.
- Some P53 hotspot mutations are in DNA binding domain and are gain of function (GOF).
- GOF p53 mutant unable to affect gene function in MML4 and MLL, resulting in upregulation of hox gene expression.
- MLL1 knockdown in GOF p53 background results in reduction of tumor growth in a mouse.
- MLL1 inhibition was synergistic with GOF p53 mutated cells.
- Inhibitors in am for targets of GOF p53…6:30
- 12:30 Resistance to mmune therapy.
- Why does it fail? Exhaustion of T cells and lack of durability.
- They evaluated the epigenetic landscape and found that PD-1 expression is high and remains high in exhausted T cells.
ATAC-seq to assess open conformation of chromatin found that in effector and naïve cells the PD-1 locus is closed but is in an open chromatin conformation in exhausted cell populations.

CARs and CARTs have seen some failure can epigenetic inhibition help?

Presented work done in collaboration with Carl June’s group, where they profiled a CLL that had a CR with CAR treatment.

The CAR integrated into the TET2 locus disrupting it catalytic function.

ATAQ-seq was run for CAR positive vs CAR negative cells. In CAR positive cells you see a reduction in ATAQ-seq peaks around IFN. GO analysis of these CAR positive cells shows genes associated with dedifferentiation of leukocytes.

The idea now is combine TET2 inhibitors with Immunotherapy: CART, CKI, and other. However, TET2 is a tumor suppressor, so not sure how this would work.

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