Calithera: A Comprehensive Review

Calithera (CALA) based out of South San Francisco, California is actively focused on developing small molecule modulators of both tumor and immune cell metabolism. The company is actively engaged in multiple trials for its lead asset CB-839, an inhibitor of the elusive target Glutaminase 1 (GLS1). So far, CB-839 appears to be much safer than prior attempts at a GLS1 inhibitor that often succumb to CNS toxicity. Further, Calithera signed a potentially lucrative co-development deal with Incyte (INCY) back in early 2017 in order to accelerate the second clinical candidate now known as INCB001158 (fka. CB-1158), a potent inhibitor of Arginase 1.

Glutaminase 1 Inhibitor (CB-839):

Below we break down each of the active trials for CB-839

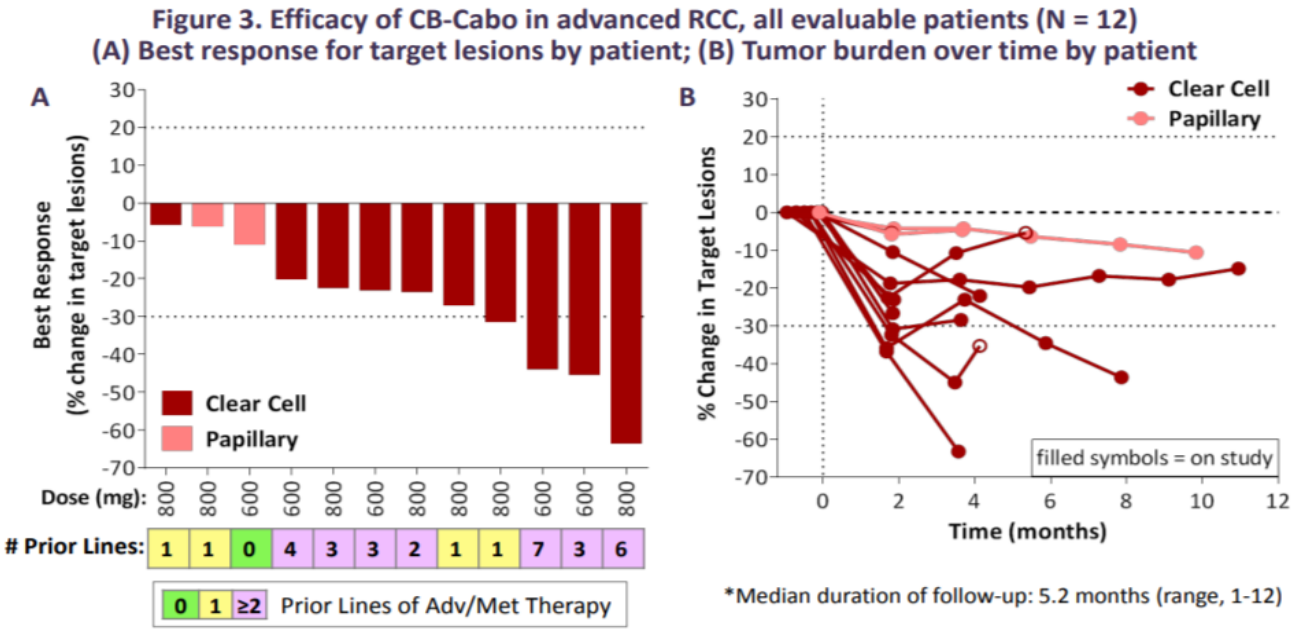
Pipeline Summary for Calithera Biosciences CB-839

Sponsor	Indication	Line	Combination Agent(s)	Phase	Est. Patients	Primary Completion	NCT ID
Calithera	mRCC	2nd-3rd	Cabozantinib	2	298	Mid-2020	NCT03428217
Calithera	TNBC	1st & 3rd+	Paclitaxel	2	112	Q3-2018	NCT03057600
NCI/Calithera	mCRC	2nd+	Panitumumab & Irinotecan	1 & 2	40	Q3-2020	NCT03263429
Calithera	ccRCC	3rd+	Everolimus	2	69	Q4-2019	NCT03163667
Calithera	Mel/ccRCC/NSCLC	2nd+	Nivolumab	1 & 2	299	Q4-2018	NCT02771626
NCI	IDHmut Astrocytoma	1st	Temozolomide & Radiation	1	40	Mid-2020	NCT03528642
Case Western	PIK3CAmut CRC	2nd+	Capecitabine	1 & 2	53	Q3-2018	NCT02861300
MDA/NCI/CALA	MDS	1st-2nd	Azacitadine	1b & 2	46	Q4-2022	NCT03047993

Note: mRCC = Metastatic Renal Cell Carcinoma, TNBC = Triple Negative Breast Cancer, mCRC = Metastatic Colorectal Carcinoma, Mel = Melanoma, ccRCC= Clear Cell Renal Cell Carcinoma, NSCLC = Non-Small Cell Lung Cancer, MDS = Myelodysplastic Syndrome

Of particular interest is the Cabozantinib (Cabo) trial in metastatic RCC, which has been designed as a blinded and placebo controlled study with potential registration. Calithera had refocused its registrational efforts in RCC from the Everolimus combination following the release of data in February of this year at the American Society Clinical Oncology Genitourinary Cancer Symposium (ASCO-GU) [1]. While the Everolimus combination did show some promise in the context of Progression Free Survival (PFS), the Cabo combination really stood apart.

Below are the spider plots for each combination presented, sourced from Calithera:



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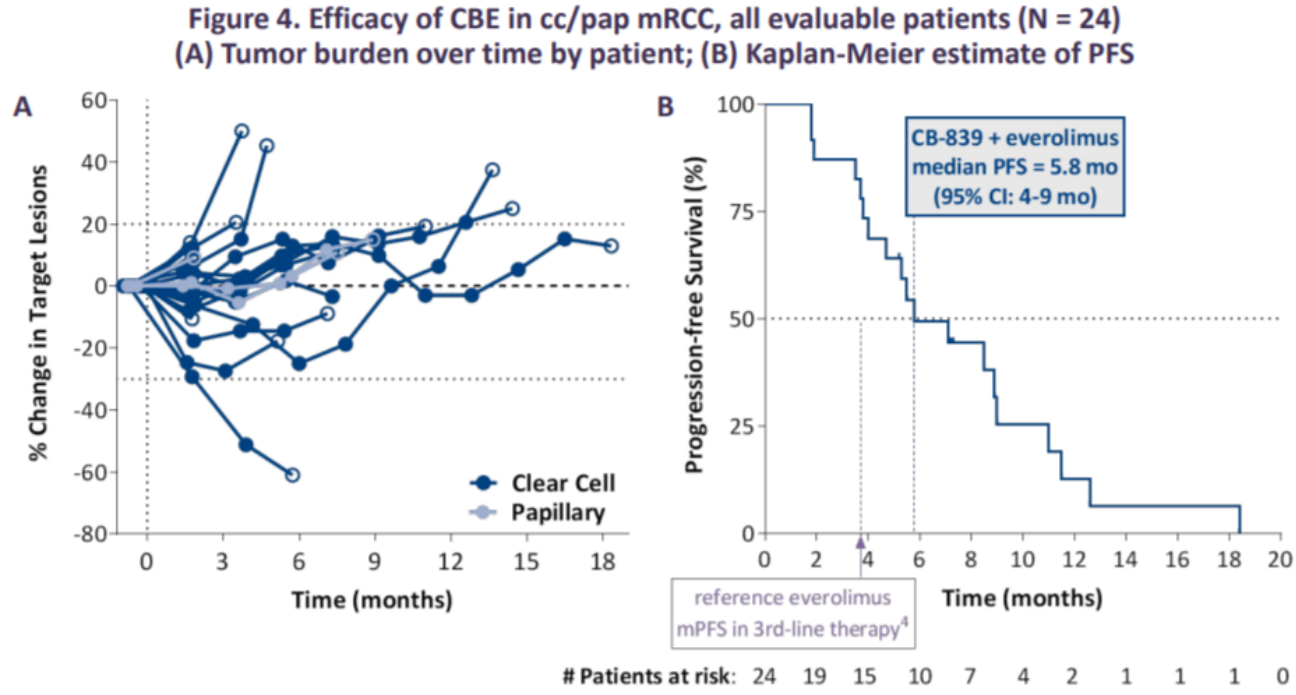


The Cabozantinib combination with CB-839 demonstrated a 100% Disease Control Rate (DCR) and a 40% Overall Response Rate (ORR) in those with ccRCC which compares favorably to the 17% ORR seen in Cabozantinib as a monotherapy in this setting. None of the subjects on this combination showed tumor growth over baseline. Notably, some of the deepest responders were also the most refracted. 3 out of 4 of the Partial Responses (PRs) came from patients who had 3+ prior therapies. 9 out of the 12 enrolled patients at cutoff remained on study (median follow up of 5.2months).

CB-839 + Cabozantinib: Exploiting Metabolic Escape in ccRCC:

In pre-clinical modelling, Calithera demonstrated the potential rationale for deeper synergy between 839 and Cabo over Everolimus [2].

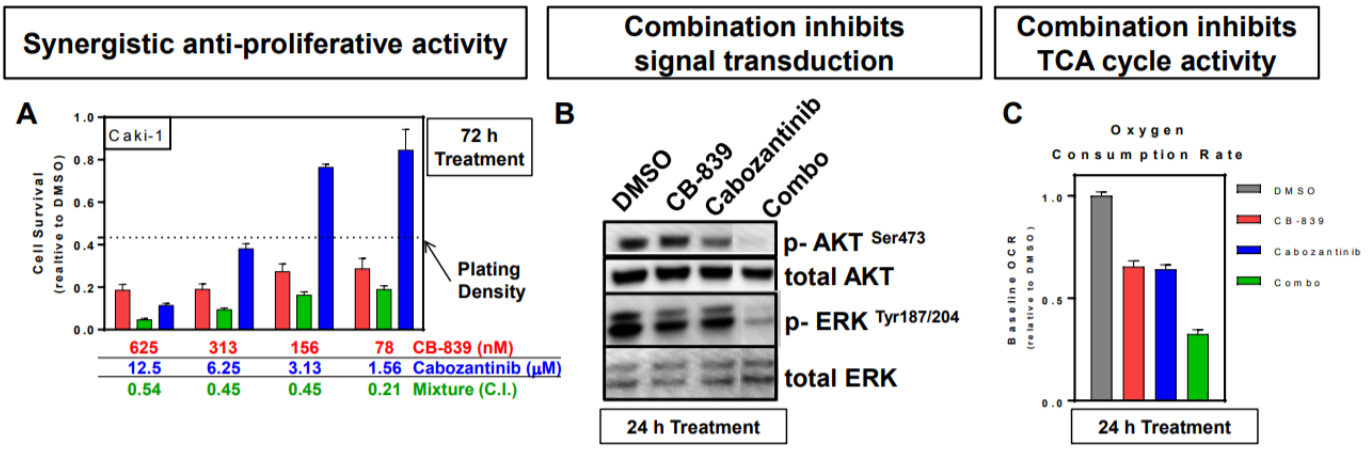


Figure 6. CB-839 synergizes with cabozantinib to decrease proliferation, signal transduction and TCA cycle activity in RCC cell line Caki-1. (A) Viability of Caki-1 cells treated with CB-839, cabozantinib or a combination of both inhibitors for 72 h. Combination Index (C.I.) was calculated using the Calcusyn Software (BioSoft). **(B)** Measurement of signal transduction in cells treated with DMSO, CB-839 (1 μM), cabozantinib (6 μM) or a combination of both drugs for 24 h and cell lysates were probed with anti-phospho-AKT and anti-phospho-ERK antibodies. **(C)** Determination of oxygen consumption rate using the Seahorse Metabolic Analyzer in cells treated for 24 hours as outlined in *(B)*.

Briefly, the western blot in figure B (a commonly employed analytic technique used to probe specific proteins in a cell sample) demonstrates that the combination of both agents appears to silence both Akt and ERK signaling. While each agent alone fails to complete downregulate each of these kinases, there is a mechanistic synergy that can starve out cancer cells.

First, Akt signaling is directly related to the mTOR axis and its activation is needed to signal the mTOR complex. Thus this combination can work upstream of mTORC1, the direct target for Everolimus therapy. Interestingly, modelling work has shown that mTORC1 inhibitors like Everolimus can augment the activation of Akt at the Ser^{473} residue, likely through mTORC2 [3]. Secondly, recent work has shown how the ERK signaling pathway is a common escape route for mTOR inhibition [4]. Considering the combination of Cabo and 839 appears to be reducing the activation of both of these pathways, it can be deduced how this combination is far more effective at starving out cancer cells than the Everolimus approach.

Clear cell tumors, which represent the vast majority of RCC histologies are characterized by von Hippel-Lindau (VHL) deficiency or mutations [5]. We particularly see value for 839+Cabo in the context of this tumor type. All RCC mutations in VHL affect its ability to manipulate the Hypoxia Inducible Factor (HIF) axis. Under healthy conditions, renal cells have a bias towards HIF1a over HIF2a whereas this is inverted in the diseased state. The low oxygen environment driven by these tumor types leads to downstream signaling that drives signaling for high levels of vascularity, expressing Vascular Endothelial Growth Factor (VEGF). In addition HIF can signal for increased mesenchymal-epithelial transition (MET) and anexelekto (AXL) signaling in RCC, all three of which are direct targets for Cabo [6].

However these signaling pathways alone are not enough for a cancer cell to survive in what would normally be highly adverse conditions. Multiple papers have demonstrated how VHL driven tumors rely on glutamine metabolism in order to survive and proliferate [7-9]. Under prolonged hypoxia, tumors exist in acidosis, a state

where the local pH is far more acidic than what one would find in healthy tissue. To survive and grow in these conditions, a significant shift occurs where tumors preferentially survive on the reductive metabolism of glutamine in the mitochondria [10]. This is a move away from utilization of glucose as a source of citrate and α-ketoglutarate (α-KG) in the Krebs cycle [11]. A-KG is required for the creation of biomass in the cancer cell as well as energy for survival [12].

Based on these findings, Calithera has recently initiated the CANTATA trial, a randomized phase 2 study evaluating the combination of CB-839 + Cabozantinib vs Placebo + Cabozantinib in patients with advanced/metastatic RCC. The patients will be randomized 1:1 into the two treatment cohorts. Further stratification will be based upon prior anti PD-(L) 1 and IMDC risk category. The primary endpoint for approval will be based upon PFS monitored by an independent radiology committee. Patients must have either failed one or two lines of prior systemic therapy including an anti-angiogenic (such as a VGFR TKI) or the combination of Nivolumab + Ipilimumab. 298 patients are estimated to be enrolled, and the study is 80% powered to show at least a 35% benefit in PFS. This falls in line with recent guidance from the FDA which primarily looks to utilize PFS as an endpoint for the Accelerated Approval (AA) pathway in oncology. Secondary endpoints will be Overall Survival (OS), ORR, Duration of Response (DOR), DCR, Safety, Pharmacokinetics, Biomarkers, and Quality of Life (QoL) measurements. Primary Completion per clinicaltrials.gov is listed as mid-2020, however the trial can be stopped early for efficacy based upon enrollment pace as well as effect size.

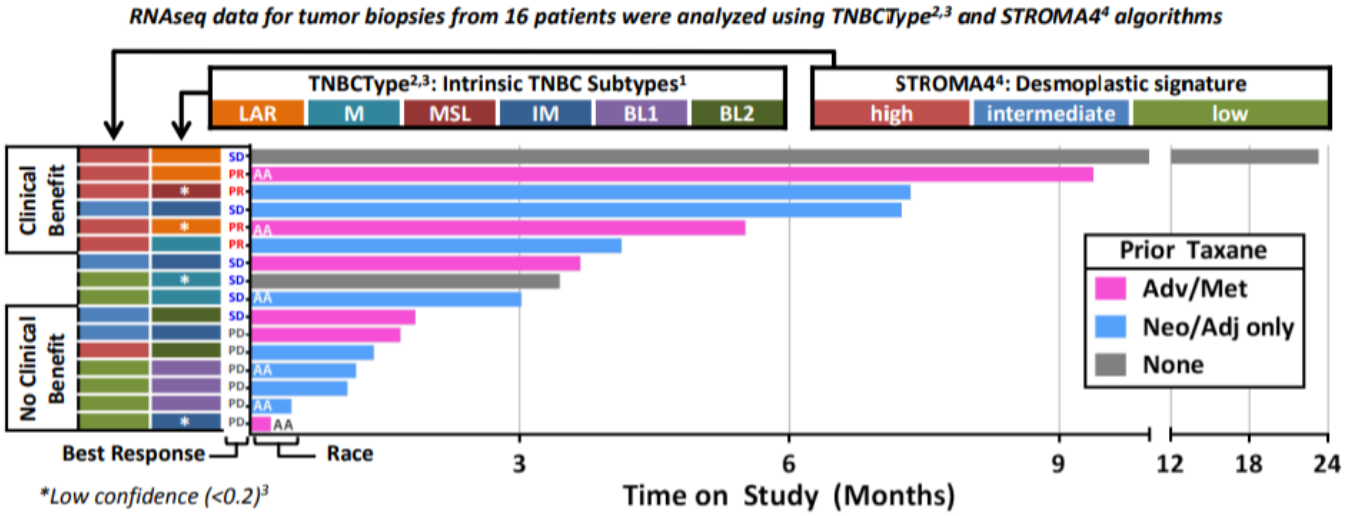
CB-839 + Paclitaxel: Reversing Resistance in TNBC:

The next major readout for CB-839 appears to be the Paclitaxel combination in both the front line and salvage settings of TNBC. Notably the trial stratifies into 4 cohorts, based upon race and line of therapy: front line African American, salvage (3rd line +) African American, and the same for non-African Americans. This is an interesting choice for a couple of reasons. Namely, African American women who are diagnosed with breast cancer, tend towards the TNBC histology at a much higher incidence than other ethnic groups. Further African American women with TNBC have a far worse prognosis [13]. Since TNBC by its very nature lacks well established hormonal targets in breast cancer such as HER2 or the Estrogen Receptor (ER), therapies typically consist of untargeted chemo and platinum therapy. New and more targeted treatment modalities are desperately needed in this setting.

One of the major oncogenes that drives metabolic transformation towards glutamine utilization by tumors is cancer associated **My**elo**c**ytomatosis (c-MYC). Much like VHL in RCC, c-MYC also appears to be directly regulated by HIF-2a across multiple tumor types [14, 15]. One study has found that MYC over expression is related to accumulation of the oncometabolite 2-hydroxyglutarate (2HG), a downstream product of glutaminase [14]. Interestingly, under normoxic conditions, the accumulation of 2HG was not nearly as severe as what is found in tumors growing under hypoxia. The study further found that cells derived from African Americans had a distinct epigenetic pattern based upon DNA methylation. This pattern was associated with a stem cell-like signature, and is not seen as frequently in other ethnic groups. Another study also supports the claim of distinct epigenetics in African American TNBC tumors, where hypermethylation was more commonly seen [17].

In the phase 1 evaluation of CB-839 treatment alongside paclitaxel in patients with advanced TNBC who had prior exposure to taxanes, 22% of patients had achieved a PR and 38% of patients with stable disease (SD). When broken down by race, the PR rate for African Americans was 27% [18]. This is an impressive result given that these patients had already refracted to this class of chemotherapy, and the response rates were higher in a

high risk population. Initial biomarkers show an interesting correlation with high expression of the Stromal axis D subtype, indicative of desmoplasia. This is characterized by increased collagens in the tumor [19]. 5/6 patients with high stromal D demonstrated disease control, 4 of whom achieved a PR.



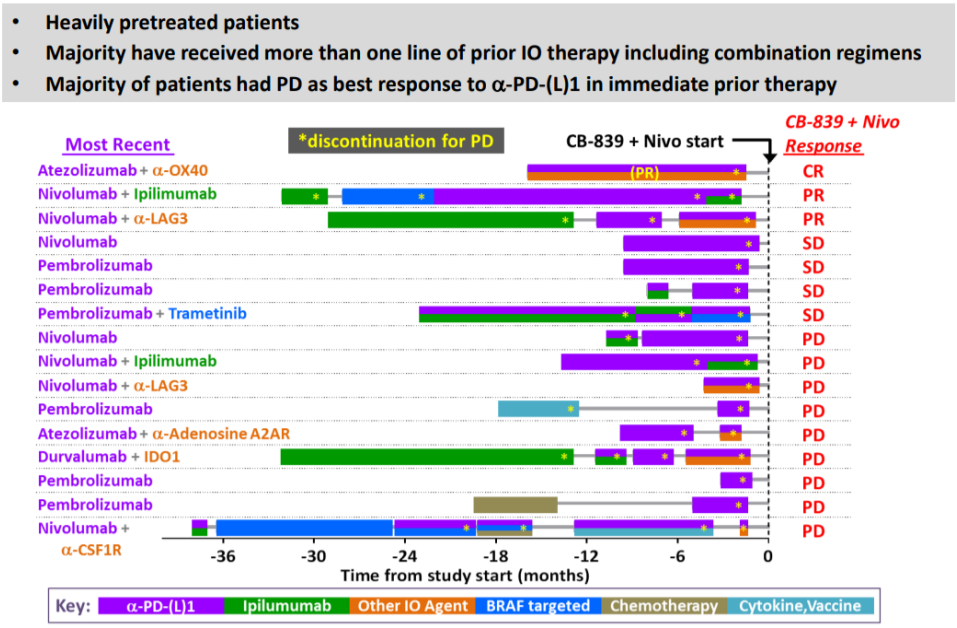
When diving deeper into the links between collagen and breast cancer, the evidence for this marker grows. One study has found that aligned collagen can result in an extremely poor prognosis independent of other factors. These researchers have found that patients are 3-4x more likely to relapse (disease free survival) [20]. Further, metabolic alterations towards glutamine dependency and away from glucose are driven by high density collagen matrices; these patients are 4-6 fold more likely to be diagnosed with breast cancer [21].

The tumor is largely cut off from the rest of the body as it builds its niche, known as the tumor microenvironment (TME). Therefore there must be a readily available source for the tumor to access glutamine. Consistent with this hypothesis, cancer associated fibroblasts (CAFs) appear to provide this fuel to the tumor under these conditions. Under conditions of starvation, CAFs increase production of glutamine from glutamine synthase — the enzyme needed to convert glutamate back into glutamine [22]. Providing evidence that tumor cells are readily dependent on these CAFs for fuel is that tumors themselves express low levels of glutamine synthase [23]. In other tumor cell types it has been observed how collagen expression leads to resistance to both chemotherapy such as taxanes and platinum therapy [24]. It should be noted that fibroblasts are the primary source of collagens. Therefore we feel that this unique profile of high levels of collagen and HIF-2a confers resistance to standard treatment and establishes a dependency on glutamine for fuel. By blocking glutaminase the tumor can no longer convert glutamine to glutamate for energy and cannot support the activity of glutamine synthase in CAFs.

CB-839 + Nivolumab in Melanoma: A Curious Complete Response:

In November of last year at the Society for Immunotherapy, Calithera announced data from the combination trial of CB-839 and nivolumab (aka Opidivo) across multiple indications (RCC, NSCLC, and Melanoma). The trial was uniquely structured in such a way that patients who were actively progressing on nivolumab were enrolled [25]. This is a departure from the standard trial design we have seen in similar contexts where patients were either PD-(L) 1 naïve (such as the Nektar (NKTR) IL-2 study [26]) or were "predicted" [27] to be resistant to treatment. Unsurprisingly this disparity led to significant confusion among market participants who attempted to make an apples to apples comparison across the trials. As a result there was a significant draw-down in equity prices for CALA, sending the stock plunging over 50%. We feel that this is an unfair evaluation of the data at hand given how unlikely it would be to see any single agent response with the PD-(L)1 class of drugs in this population. Of the combination trials, it appears that Calithera utilized the most rigorous design.

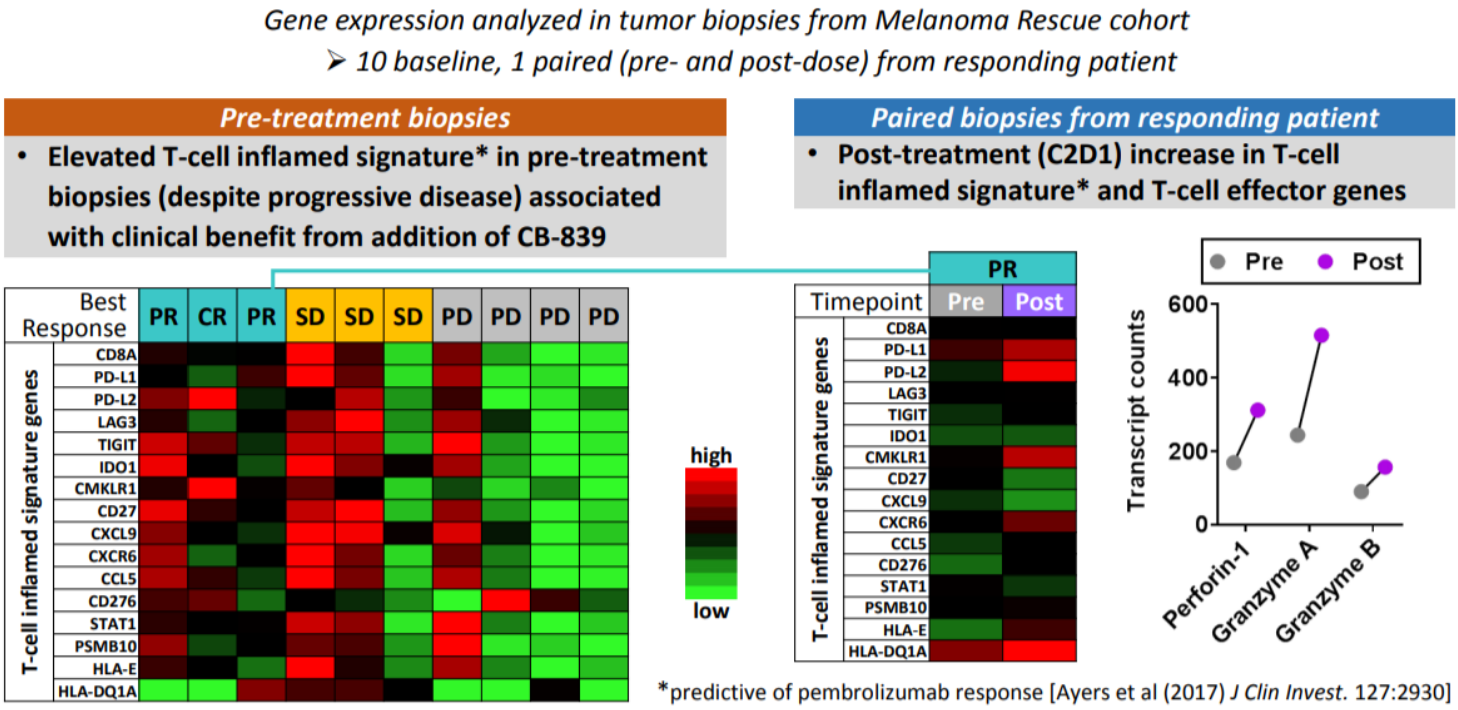
Of the 16 evaluable melanoma patients who were progressing on a checkpoint inhibitor at study entry, we saw one CR and two PRs for an ORR of 19% and a DCR of 44%. We particularly want to focus on the one CR seen in the study which was later revealed on the associated conference call to be desmoplastic. This is an interesting finding given what we established earlier in TNBC that 4/6 patients with high levels of the Stromal D signature achieved a PR. Considering that both TNBC and Melanoma are both tumor types with cutaneous or near cutaneous lesions, it would be relatively easy for investigators to conduct multiple biopsies in order to determine how these biomarkers evolve with treatment.



Melanoma by its nature has a high level of collagen expression, and monitoring how patients respond over time in this setting can reveal a significant amount regarding where to develop the treatment. Given that it appears sensitivity to CB-839 is driven by a unique metabolic signature as well as TME structure, establishing this profile can drive an indication agnostic approval down the line.

It's important for investors to realize that a main goal of this study is to marker out how CB-839 can impact immune cell metabolism. Even though the drug was not designed to modulate t-cell metabolism, it does appear that there is activity in this regard. To this ends, Bristol-Myers Squibb and Calithera further expanded their collaboration in this trial for the melanoma cohort to expand enrollment. "As part of the expanded collaboration, melanoma development costs will be shared, and a joint development committee will be established to guide the development and regulatory strategy".

One possible theory for this observation is that glutamine is required by T-cells and has a key role in maintaining effector cell fitness and viability [28, 29]. However this effect does require T-cell Receptor recognition of a tumor associated antigen (TAA) and subsequent activation. In other words, high levels of glutamine available to a T-cell in itself will not do much good unless the T-cell can recognize the tumor. Absent of PD-1 blockade it is difficult for T-cells to utilize glutamine or glucose as fuel [30]. When looking at the biomarker data provided by the company related to how "hot" the tumors were at study entry, we can clearly see that colder phenotypes are unlikely to respond. CB-839 appears to be an agent which can take an immunogenically "warm" tumor and turn it hotter, but will have minimal impact to cold tumors.



In the other treatment cohorts, we saw some evidence of activity. Among six evaluable NSCLC patients four achieved a SD. For the RCC rescue cohort, eight patients were evaluable and six achieved a SD. This may not seem significant, but both of these tumor types are immunologically colder than Melanoma and thus consistent with the hypothesis. Also notable is the fact that safety appears in line with Nivolumab alone, offering a low risk option for combination.

Arginase 1 Inhibitor (INCB001158):

In Janurary of 2017, Incyte (INCY) signed a co-development deal with Calithera to rapidly expand and accelerate CB-1158, now known as INCB001158, through the clinic. As part of the deal economics, Calithera received $45 million in an upfront payment as well as an equity investment of $8 million in shares at $4.65. Both companies will jointly co-fund the remainder of clinical development activities, 70% by Incyte and 30% by Calithera. Further, Incyte and Calithera will split the profits and losses from commercialization at a ratio of 60/40 respectively in the United States. Calithera is entitled to over $430 million dollars in future milestones if the company chooses to continue to co-fund, or can opt out of these obligations to receive up to $750 million in milestones. Outside the US, Calithera will be eligible to receive unspecified tiered royalties ranging from the "low-to-mid double-digits" [30].

Importantly, Calithera retains the rights to the use of arginase inhibitors outside of oncology as well as internal combinations across indications. Recently the company has announced plans to bring forward arginase inhibitors for pulmonary indications, starting with an IND application for cystic fibrosis (CF) in the second half of this year. Accordingly, Incyte has the right to negotiate a deal for this and other indications outside of oncology, but would require an expansion of the original deal. We see any deal for CF as indicative of success in the ongoing trials in oncology, particularly NSCLC and Mesothelioma. Below we break down the open trials evaluating the activity of INCB001158 as a single agent and in combination.

Pipeline Summary for INCB001158 Co-Sponsored with Incyte

Combination Agent	Indication	Prior Treatment	Study Start	Completion	NCT
None	NSCLC (EGFR, ALK-) CRC Solid Tumors	SOC	Sep-16		
Pembrolizumab	NSCLC (EGFR, ALK-) Melanoma Urothelial MSI CRC	anti-PD-1 immediately prior (PD or prolonged SD)	Oct-17	May-19	NCT02903914
	MSS CRC	CPI naïve, prior 5-FU			
	Gastric SCCHN	CPI naïve			
	Mesothelioma	R/R or Intol to 1st line, CPI naïve			
FOLFOX Gem/Cisplatin Paclitaxel	BTC, CRC, Endometrial, GC, Ovarian, Solid Tumors	Any (None Specified for Exclusion)	Nov-17	Q2-20	NCT03314935

Note: EGFR = Epidermal Growth Factor Receptor, ALK = Anaplastic Lymphoma Kinase, MSI = Microsattelite Instable, MSS = Microsattelite Stable, SCCHN = Squamous Cell Carcinoma of the Head and Neck, BTC = Biliary Tract Cancer, GC = Gastroesophageal Cancer

In the next sections we dissect how arginase plays a key role in modulating the immune system by its compartments. Further, we discuss how there remains significant opportunity for Calithera to explore in-house combinations and future directions. Lastly, we elaborate why success in an indication such as lung cancer or mesothelioma is crucial for future applicability across the pulmonary space.

Arginase Expression in Myeloid Derived Suppressor Cells (MDSCs) and Tumor Associated Macrophages (TAMs):

Numerous studies have demonstrated how MDSCs and alternatively activated macrophages (M2) are associated with poor prognosis across multiple cancer types, particularly through immunosuppression [31-33]. Under healthy conditions these cells play a key role in wound healing, however the tumor hijacks this mechanism in order to build a niche in which it can thrive. Macrophages are extremely flexible phenotypically, switching between the classically activated state (M1) which is pro-inflammatory and the suppressive M2 state. A key mechanism by which these cells exert their immunosuppressive capabilities is through the production of arginase.

There is competition for the catabolism of arginine between the isoforms of Nitric Oxide Synthase (NOS) and arginase. All NOS enzymes process arginine to nitric oxide, necessary for a cytotoxic response. Of particular interest in the context of immunotherapy is the NOS2 isoform, which is induced by inflammatory cytokines like interferon γ (IFNγ), tumor necrosis factor α (TNFα), and IL-1β. M1 macrophages are the primary sources of NOS2 when responding to diseased cells [34-37].

NOS2 is far more potent at metabolizing arginine than arginase (the arginine Km of NOS is around 3 μM whereas arginase is approximately 2mM [38]). However the interplay of these two enzymes is a bit more complicated than it would first seem. Arginase has the capacity to uncouple NOS. The uncoupled variant of NOS produces far less cytotoxic nitric oxide and consumes more oxygen in order to form the free radical peroxynitrite [39]. One study found that peroxynitrite is a direct mechanism for resistance to immunotherapy as mediated by MDSCs to modify cytotoxic t-cell activity [40].

According to data presented by Calithera, which used immunofluorescence to determine the expression of arginase 1, it became clear that the majority of the enzymes comes from MDSCs. Further, Arginase is expressed across multiple tumor types, predominately lung and GI cancers making it an attractive target for inhibition [41].

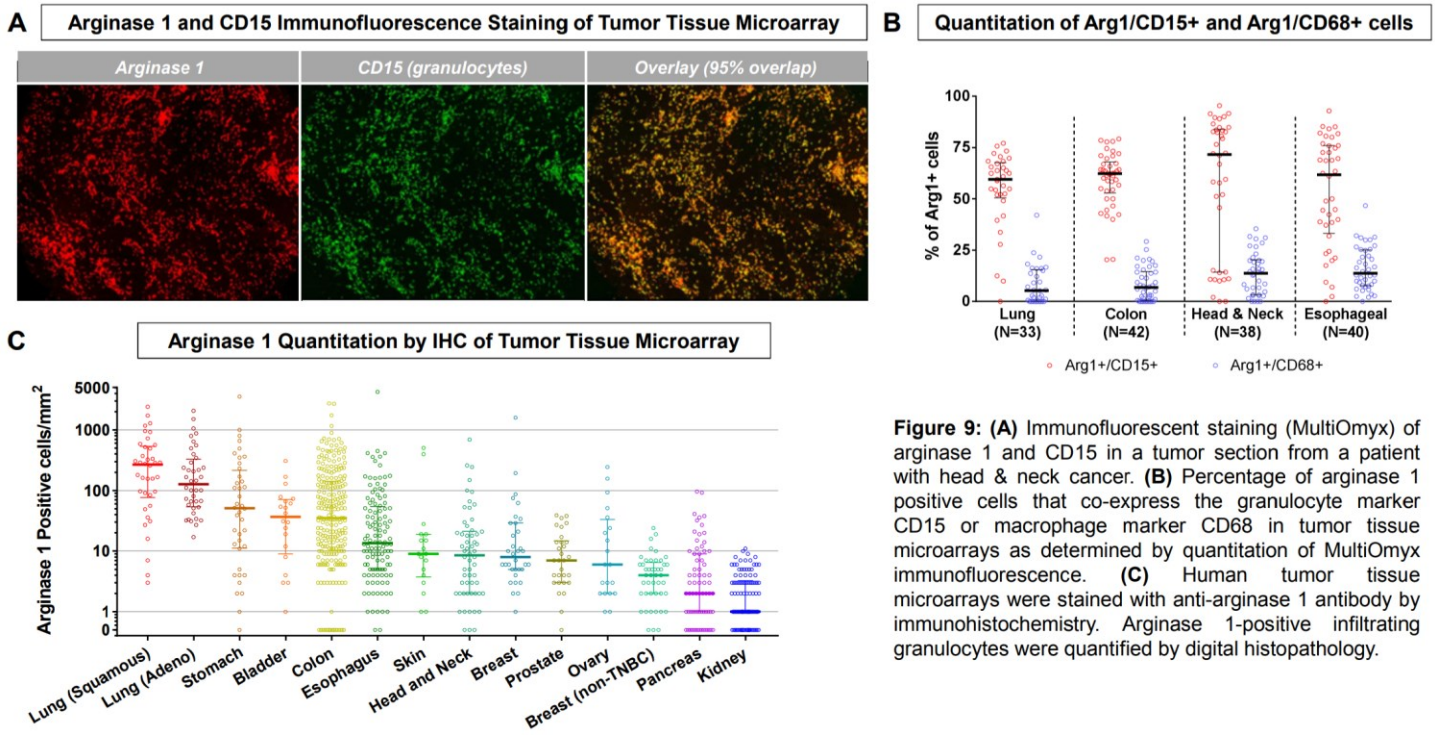


Figure 9: (A) Immunofluorescent staining (MultiOmyx) of arginase 1 and CD15 in a tumor section from a patient with head & neck cancer. **(B)** Percentage of arginase 1 positive cells that co-express the granulocyte marker CD15 or macrophage marker CD68 in tumor tissue microarrays as determined by quantitation of MultiOmyx immunofluorescence. **(C)** Human tumor tissue microarrays were stained with anti-arginase 1 antibody by immunohistochemistry. Arginase 1-positive infiltrating granulocytes were quantified by digital histopathology.

The Arginase-IDO pathway in Dendritic Cells (DCs) and the By-Products of Arginine Metabolism:

When metabolized by arginase, arginine produces ornithine and urea. Ornithine (Orn) in particular is of key interest as it is a major building block for collagens that can help the tumor grow resistant to treatment [42]. Orn can be further transformed into sperimidine and finally spermine by Orn decarboxylase (ODC). These polyamines have been recently implicated in promoting IDO1 signaling in DCs, likely through the activation of the Src kinase. However, depletion of arginine itself does not appear to cause induction of the IDO1 enzyme [43]. This supports the notion that arginine when metabolized by arginase, but not the NOS family supports an immunosuppressive environment.

Some of the key markers in patients with various cancers we often find elevated are serum levels of IL-10 and TGF-β [44]. In DCs which are stimulated with TGF-β, IDO1 becomes phosphorylated in a self-perpetuating loop that leads to longer lasting immunosuppressive effects. However, absent of arginase signaling it appears that this process of IDO1 induction fails to take hold. Further diving into this mechanistically, it appears that arginase induction occurs well before the expression of IDO in DCs [43].

One may assume that much of the feedback loop that drives consistent IDO expression is the presence of the downstream polyamines of arginase metabolism. Consistent with this notion is the observation that blocking these products appears to induce greater levels of T-cell activity [44]. Another study which looked to inhibit ODC observed that the suppressive effect of MDSCs can be largely silenced through this mechanism [45]. In particular the study not only saw a loss of arginase expression, thus breaking the feedback loop, but also the adenosine pathway.

Dendritic cells themselves can be producers of the arginase enzyme. Il-6 is a direct target of HIF-2α, and has been shown to induce expression of arginase in DCs [45-47]. Given that we know arginase can deplete local levels of oxygen, and thus induce a hypoxic state this is an interesting feedback mechanism unto itself. It can be hypothesized that not only the oxygen depletion, but also the Orn derivatives in building out the tumor niche leads to consistent over expression of arginase.

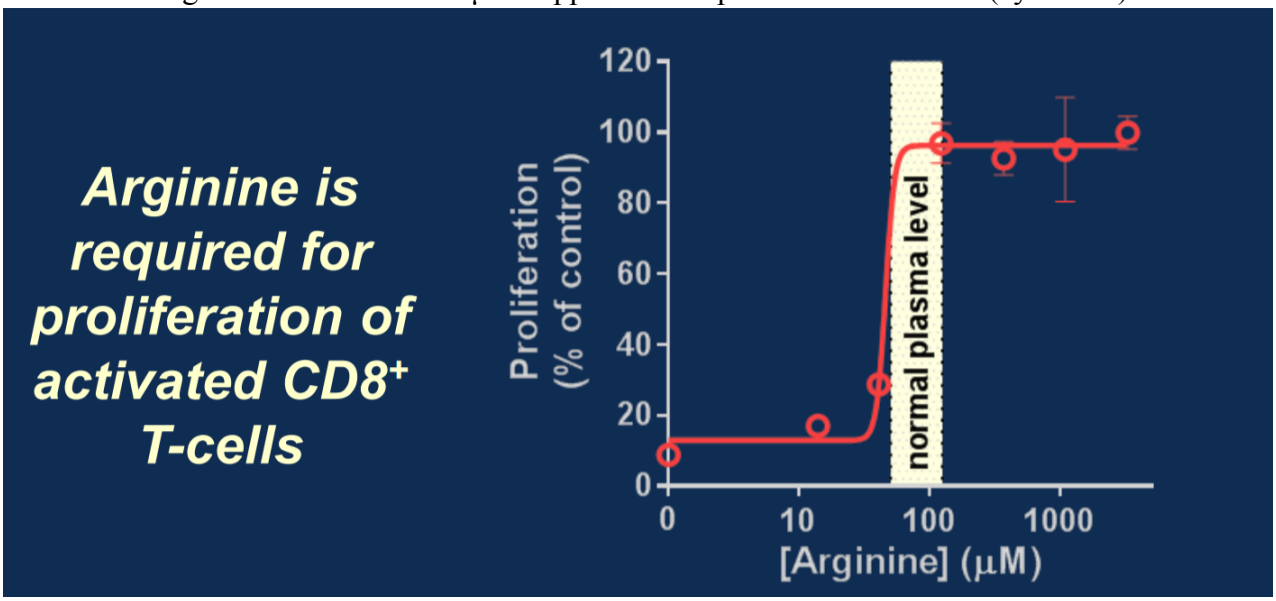
Some tumors themselves, such as lung adenocarcinoma, can produce IL-6 [48]. Unsurprisingly, this is consistent with Calithera's findings that lung tumors are among the highest expressers of arginase. This cytokine can significantly inhibit the maturation of DCs via the STAT3 pathway as well as prevent their expression of MHC-II, needed to train T-cells [49]. In addition it was found that in MDSCs, STAT3 is a direct regulator of arginase expression as multiple binding sites for STAT3 were found in the arginase promoter region [50]. Since arginase appears to be upstream of IDO expression in DCs, breaking the tolerance at this level may be far more effective than direct inhibition of IDO.

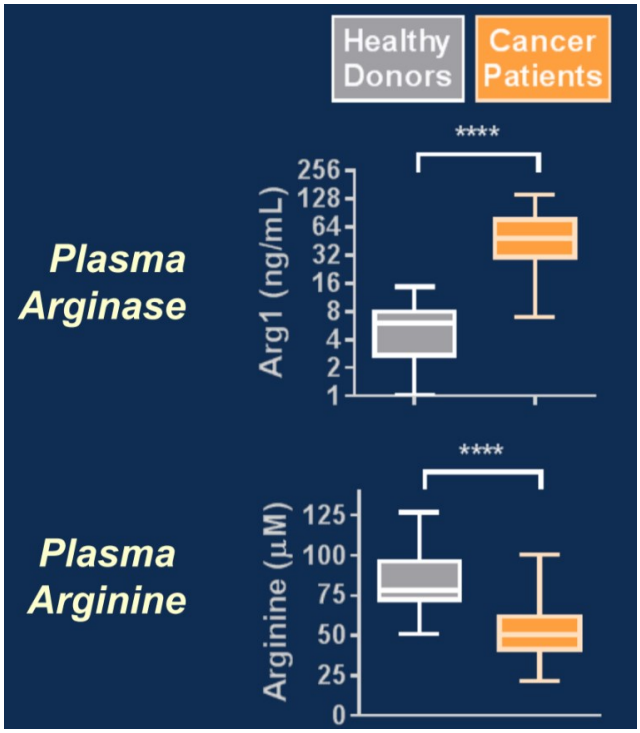
Recently, significant doubts have been raised in regards to the utility of IDO inhibition in cancer. This is largely due to the failure of Incyte's epacadostat program to demonstrate PFS survival benefit, despite compelling earlier stage results. When diving into the details we notice that the initial trial results from ECHO-202 which looked promising for melanoma, was largely in front line patients. Only 17% of the patients which had been enrolled had 1 line of prior therapy for advanced disease. The standard of care for melanoma outside of IO therapy (since prior IO was excluded) is to utilize BRAF inhibitors [50]. In the registration design however, Incyte opted to include those who had relapsed from a BRAF inhibitor. Resistance to BRAF therapy, in the form of mutations, appears to drive downregulation of IFNγ [51]. 44.5% of patients enrolled in the ECHO-301 trial were in fact BRAF mutant [52]. This flies in the face of the proposed mechanism of action for IDO, which was initially found to be induced by IFNγ [53]. We now know that the downstream products of arginase are also quite effective at inducing IDO1 activation, suggesting that arginase is likely the escape mechanism. Failure of Incyte to establish a biomarker in the ECHO-202 is also a likely contributor to the program's termination.

The Role of Arginase in T-Cell and NK-Cell Activity:

Arginase plays a multifaceted role in the modulation of T-cell activity. One of the best described mechanisms in literature is via the regulation of the CD3ζ Chain [54-59]. In order for a T-cell to respond to a recognized TAA, a functional T-cell Receptor (TCR) is required. The zeta chain is essential to couple this recognition with intracellular signaling pathways such as the Janus kinase 3, necessary for clonal expansion [60]. Immunological memory is dependent of this clonal expansion and responsiveness of antigen-specific T-cells [61].

However, the suppressive impact of arginase can be quickly reversed and experienced T-cells can reactivate in the presence of sufficient arginine availability [62]. This notion is supported by Calithera's own pre-clinical work that demonstrates how arginine can function as a metabolic switch for the CD3 receptor [63,64]. The company has shown how arginine levels below 40µM suppresses the proliferation of CD8 (cytotoxic) T-cells.





Further, Calithera has demonstrated how plasma levels of arginine in cancer patients are often much lower than in healthy samples and below this threshold. One can safely assume that within the TME, these levels are even more suppressed.

Arginine metabolism doesn't just play a role in re-activating T-cells, but also seems to have a major impact on the quality and durability of T-cells generated. Metabolomic and proteonomic analysis of cultured T-cells and in-vivo have shown how elevating arginine levels induced a shift from glucose metabolism and supported the development of memory subtypes [65]. Naïve T-cells typically require very little nutrient uptake and rely predominately on oxidative phosphorylation (OXPHOS), but upon antigenic stimulation their requirements rapidly shifts towards glycolytic and glutaminolytic activity [66-68]. However, once finished with the immune response most of the expanded T-cells die off, also known as apoptosis.

Based upon the metabolomics study referenced, T-cells rapidly uptake arginine and within 24-48 hours. This traced arginine was rapidly metabolized by arginase 2, another isoform of this enzyme found in mitochondria. Like arginase 1, arginase 2 broke down the arginine into useful building blocks for the t-cell like ornithine and proline. However since this enzyme and the arginine itself was localized within the T-cell, the surrounding cells are unable to access the material. Interestingly, the authors found that arginine uptake by T-cells exceeded the necessary amount for protein synthesis by over 2-fold. Following this significant uptake of arginine, it was noted that the now experienced t-cells switched back from utilization of glycolysis for fuel towards mitochondrial OXPHOS.

When T-cells are activated in environments with high level of arginine, they aren't nearly as inflammatory as in standard medium, as measured by IFNγ production. Yet these cells can be easily reactivated upon secondary stimulation and produced even more IFNγ than primarily activated T-cells in the presence of glucose. This property is characteristic of Central memory CD8+ T-cells (Tcm). Even absent of persistent cytokine stimulation, the presence of enough arginine in the Tcm subtype was enough to maintain their survival and train the immune system. Also, Tcm cells have been shown to be far superior to effector memory CD+8 T-cells (Tem) in eliminating tumors. Terminally differentiated T-cells appear to have minimal viability and tumor killing capacity [70,71]. It appears that glucose based metabolic activation of T-cells will not be sufficient in generating durable response, absent of arginine.

This may explain some of the confusion around the Nektar dataset for pegylated-IL2. Not only has it been discovered that IL-2 stimulation can cause an induction of arginase activity by MDSCs [72], but also supports a metabolic shift towards glycolysis [73, 74]. While initial responses appeared promising, durability is now largely in question sending shares down by more than 50%. Arginase inhibition maybe necessary to rescue relapsed patients from IL-2 treatment failure.

Arginase may also play a key role for the development of other T-cell targeted therapies. It appears that CAR-T responses which are more persistent are correlated with a bias towards OXPHOS and mitochondrial biogenesis. These cells also show markers of the Tcm phenotype consistent with the findings of the previously referenced work [75]. Considering that CAR-T and TCR therapies struggle with efficacy in solid tumors, one major explanation for this could be related to the presence of MDSCs which express arginase in the tumor niche. Both of these modalities rely on a functional CD3ζ complex and require persistence in order to generate lasting regressions. Further optionality for the company to combine INCB001158 exists for CD3 engaging bispecific antibodies. Notably, Incyte struck a deal with Merus (MRUS) to gain access to a portfolio of bispecific antibody targets [76]. This could be an area for the collaboration to expand once the initial model for arginase inhibition proves in human.

In Calithera's own modelling, INCB001158 was more effective than T-cell therapy alone and also showed a strong synergistic effect. Also notable was the efficacy appears to be similar to gemcitabine as a single agent in lung and colon cancer models. The therapy also increases NK cell populations, which upon activation can support T-cell responses and antigen presentation. Two rationales exist for this observation. First, in the presence of arginase, NK cannot easily produce supportive cytokines such as IL-12 and IL-18 [77]. Second, arginine allows DCs to mature and thus can produce IL-15 [78]. This unique cytokine not only helps to expand NK cell populations but also helps to regulate memory T-cells [79, 80]. Finally, NK cell proliferation is directly controlled by the availability of arginine [81]. We can see the impact of this shift in the populations of lymphocytes in preclinical modelling.

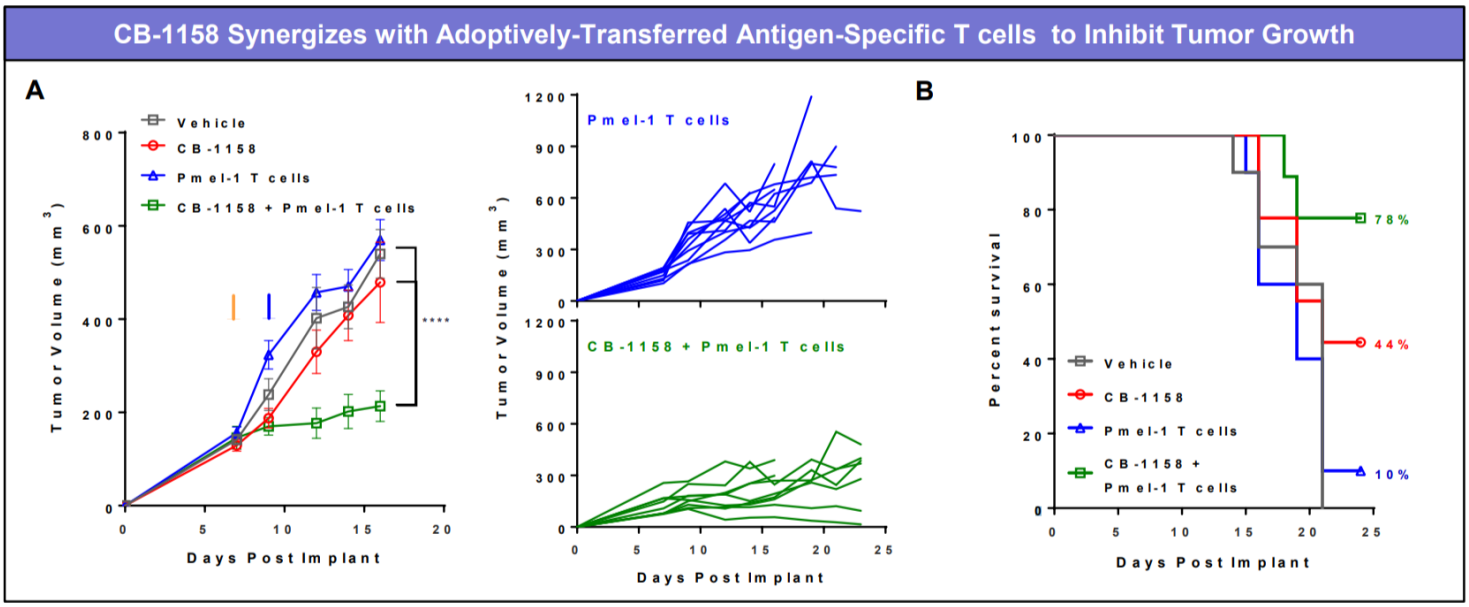


Figure 4: (A) B16F10 cells were implanted in C57.Bl/6 mice. Non-myeloablative chemotherapy (Cyclophosphamide 250 mg/kg and Fludarabine 50 mg/kg) was dosed IP on day 7 (orange arrow) to all groups. Pmel-1 CD8 T cells (1 x 10^6) were adoptively transferred IV on day 9 (blue arrow). Recombinant human IL-2 (200,000 UI) was dosed IP BID on days 9, 10 and 11 in mice receiving T cells (no effect on tumor growth; data not shown). CB-1158 was dosed at 100 mg/kg PO BID starting on day 2. (N = 9-10 per group; **** P < 0.0001). **(B)** Survival curves. P = 0.0137 by Mantel-Cox test.

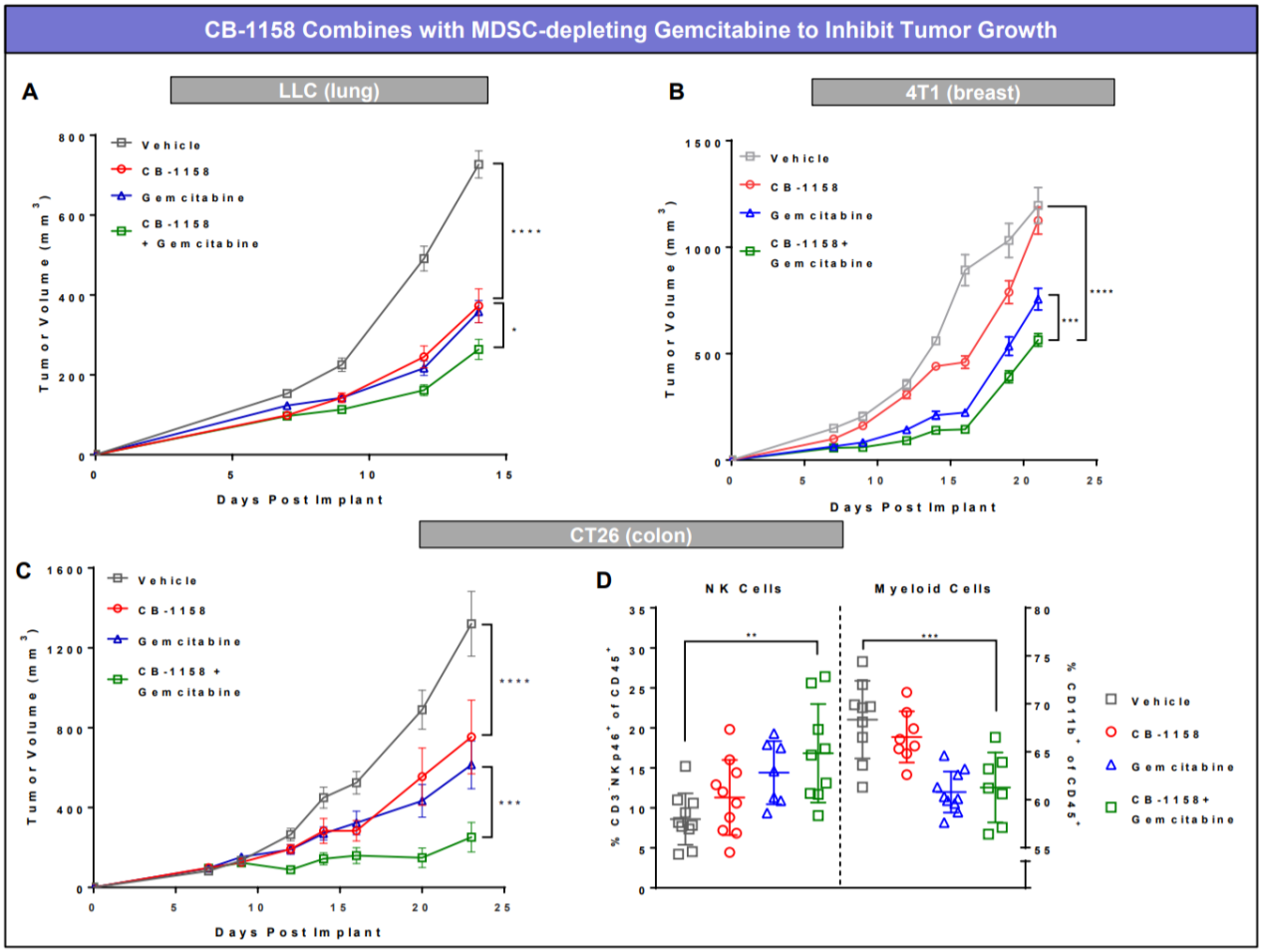


Figure 7: (A) LLC cells were implanted in C57.Bl/6 mice. CB-1158 was dosed at 100 mg/kg PO BID starting on day 2. Gemcitabine was dosed at 60 mg/kg IP on days 6 and 10. **(B)** 4T1 cells were implanted orthotopically into female Balb/c mice. CB-1158 was dosed at 100 mg/kg PO BID starting on day 2. Gemcitabine was dosed at 30 mg/kg IP on day 5. **(C)** CT26 cells were implanted in Balb/c mice. CB-1158 was dosed at 100 mg/kg PO BID starting on day 2. Gemcitabine was dosed at 50 mg/kg IP on days 10 and 16. **(D)** Levels of immune cell subsets determined by flow cytometry in CT26 tumors from mice treated as in **(C)** for 13 days.
(N = 10 per group; **** P < 0.0001; *** P < 0.001; ** P < 0.01; * P < 0.05).

Considering that nutrient availability appears to drive the ability for immune cells respond, we feel that retaining the right to internal combinations gives Calithera a unique leverage optionality. In the tumor microenviroment, glutamine and glucose are both starved which makes it difficult for T-cells to initiate a response. Lack of arginine makes it difficult for activated T-cells to maintain this response. As such a combination approach of both inhibiting glutaminase and arginase may provide a potent means to shift the polarity in the TME. Since many of the factors which sensitize tumors to treatment are driven by hypoxia as well as ornithine derivatives such as collagen, such an approach has the capacity to remodel the TME. It should be noted that glutamate, the product of the enzymatic reaction of glutaminase and glutamine is directly needed for ornithine synthesis. By restructuring the tumor, and starving out cancer cells from both the Krebs cycle and the Urea cycle, we could see a larger immune infiltrate into the tumor. This has the potential to turn cold tumors hot again. But also, this effect may have an interesting effect on lung fibrosis. Early data from the 839 program needs to be taken into context for the larger picture.

A Future Direction in Lung Fibrosis:

Many of the hallmarks of pulmonary fibrosis share similarities with that of lung cancer. Persistent fibrosis is often characterized by proliferation of fibroblasts and collagens [81]. Interestingly there is growing evidence for a link between chronic obstructive pulmonary disease and the development of lung cancer [97].Along these lines, markering studies have revealed that arginase activity is dramatically increased in patients with Idiopathic Pulmonary Fibrosis (IPF) [82]. Further, the levels of arginase are directly correlated with the progression of IPF [83]. Consistent with this observation, MDSCs also reflect the disease status in IPF and can be used a powerful biomarker for this disease that lacks treatment options [84]. This study found that circulating MDSCs had inversely correlated with maximum vital capacity in IPF with a very strong signal ($r=-0.48$, $p\leq0.0001$). Further, it was discovered that T-cell signals were significantly blunted in these patients [85]. Considering that MDSCs are the primary sources of arginase expression, these observations do not come at a surprise.

In terms of CF, a key marker of disease progression is impairment of exhaled Nitric Oxide (NO) levels [86]. As a reminder, NOS is the primary source of NO and directly competes with arginase. There is growing evidence that these low levels of NO can increase the susceptibility to infection in these patients, one of the most common complications in CF [87]. To this ends, attempts have been made to either replace NO through inhaled therapy, or to increase levels of arginine.

Inhaled NO has shown some benefit for patients, reducing bacterial counts and reversing the characteristic hypoxic pulmonary vasoconstriction [88-90]. However some conflicting reports suggest these benefits are largely muted [91]. No large randomized trial has demonstrated data for benefit from NO inhalation, presumably because this approach works largely downstream of the underlying dysfunction. Studies evaluating inhaled arginine showed some marginal benefit, but again we see a mixed bag of results. Without shifting the polarity from Arginase to NOS, it's difficult to see how this approach would yield meaningful results. One study revealed that arginine supplementation resulted in a marginal increase in NO, but no impacted on expiratory volume [92]. However another study noticed no impact from this approach on NO [93]. The strongest signal came from a randomized trial in 19 patients which demonstrated a benefit but not statistically significant improvement in forced expiratory volume in one second (FEV1) [94].

Perhaps a combinatorial approach of inhibiting glutaminase and arginase will show a greater benefit. Mylofibroblasts are a key mediator in the progression of fibrosis. It was recently revealed that a second mechanism for TGF-β induced fibrosis is directly via the glutaminase pathway [95]. As a reminder collagen production is dependent on both arginine byproducts and glutamate. Vascular stiffness and contraction is a primary driver of almost all fibrotic pathologies, especially in lung. It was recently found that in the context of pulmonary hypertension (PAH), glutaminase activity is directly implicated in the development of the disease.

These independent researchers tested CB-839 on a model and found a significant benefit, helping to corroborate the value of glutaminase inhibition outside of cancer as well [96].

It appears that based on the optionality to negotiate an expansion deal for arginase, Incyte would look towards Mesothelioma and lung cancer data in arginase as a go/no go signal on validity of this approach overall. If proven, then a novel angle based on glutamate and arginine derived ornithine and polyamines is established to treat these rather large unmet needs. Mesothelioma is a highly difficult to treat condition and shares many similarities with PAH and lung fibrosis. Timing would suggest that the IND for arginase in CF would occur prior to a data release from arginase inhibition in lung cancer and mesothelioma. Therefore a deal here would give us a strong signal on the success of these oncological lung indications.

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