PROTOCOL

TITLE: Phase I/II Dose Escalation and Cohort Expansion of Safety and Tolerability Study of Intratumoral CD40 Agonistic Monoclonal Antibody APX005M in Combination with Systemic Pembrolizumab in Patients with Metastatic Melanoma

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Protocol Amendments

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Version 8 – 01 February 2024	Amendment 7	

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ABSTRACT

Novel immunotherapies have transformed the treatment of metastatic melanoma. Checkpoint blockade modalities like ipilimumab (anti-CTLA-4 antibody) and PD-1 blocking agents have significantly enhanced overall survival. However, long-term survival and durable remission rates remain low and new treatment options are needed to improve clinical outcome. Checkpoint blockade therapies enhance the anti-tumor immune response through inhibition of negative signaling in already existing and spontaneously induced tumor-specific T-cells. However, many tumors may not be immunogenic enough to induce such specific T-cells, which is consistent with the relatively low response rate of these modalities, in particular ipilimumab.

In situ vaccination or utilizing the patient's own tumor as a "vaccine site" through direct intra-tumoral (IT) immune modulation will lead to activation of dendritic cells (DCs) and subsequent T-cell stimulation, which can generate a specific anti-tumoral immunity. Combining this strategy with systemic immune stimulation such checkpoint blockade could lead to specific and long lasting anti-tumor immune response that may be translated into higher clinical response and durable remission rates.

There is growing body of evidence supporting the crucial role of CD40 activation for full maturation and activation of DCs that can promote antigen/tumor specific T-cell responses and generate potent anti-tumor response. Indeed, our preclinical data demonstrated that IT treatment with recombinant adenovirus encoding the dendritic cell-activating CD40L induced systemic anti-tumor effects, mediated by CD8+ T-cells. Furthermore, these tumor specific T-cell responses were further enhanced when the IT CD40L treatment was combined with systemic injection of anti CTLA-4 or anti PD-1, which led to distant tumors eradication.

APX005M is a CD40 agonistic monoclonal antibody (mAb). In vitro, upon binding to CD40, APX005M activates the CD40 signaling pathway, leading to activation of DCs. In vivo, APX005M demonstrated potent anti-tumor activity in multiple human tumor xenograft models.

We hypothesize that local innate immune activation through IT administration of a CD40 agonistic mAb can lead to enhanced induction of tumor-specific CD8+ T-cells and synergize with systemic therapies based on PD-1 blockade, resulting in superior anti-melanoma activity. This phase I/II trial evaluates the safety and immunological impact of IT administration of CD40 mAb APX005M in combination with intravenous (IV) administration of pembrolizumab in patients with metastatic melanoma. The immune response in both injected and non-injected tumors is evaluated. This trial may help in the generation of principles that can be applied to the treatment of other common cancers.

1 BACKGROUND & RATIONALE

1.1 MELANOMA

Metastatic melanoma (MM) is a challenging disease with very poor prognosis. It is estimated that there will be around 76,000 new cases of melanoma with 9700 deaths in 2014 [1]. The incidence of invasive melanoma in the United States continues to increase approximately 4-6% annually despite efforts to improve primary prevention. Additionally, melanoma survivors are 9 times as high as the general population to develop additional melanomas due to genetic risk factors and/or overexposure to ultraviolet radiation [2]. However, the landscape of therapy of metastatic melanoma is rapidly progressing. Recently, new drugs have been approved by FDA. Ipilimumab, a monoclonal antibody targeting cytotoxic T-lymphocyte–associated antigen (CTLA-4), has been shown to improve median survival of MM patients in randomized trials[3]; however, response rates are still under 11%[3]. By contrast, oncogene-targeted therapy with the small molecule BRAF inhibitors vemurafenib, debrafenib and trametinib has shown high response rates in metastatic melanoma patients harboring BRAF mutations. However, these responses are transient owing to the frequent development of resistance to these drugs.

Additionally, only around 50% of patients carry the BRAFV600E mutation sensitive to these targeted agents. Immunotherapeutic agents against PD-1 have been approved with higher response rates than ipilimumab[4-7]. Yet durable response remains in small fraction of patient. In summary, metastatic melanoma remains largely fatal disease, and the 5-year survival rates for distant stage diseases are 15% [8].

1.2 RATIONALE FOR COMBINATION OF SELECTIVE LOCAL INNATE IMMUNE ACTIVATION WITH SYSTEMIC CHECKPOINT BLOCKADE IMMUNOTHERAPY OBJECTIVES

Melanoma is one of the most responsive cancers to immune-based therapies; particularly those mediated by cytotoxic T-lymphocytes (CTL), and melanoma represents a good model system that may lead to immune-based therapies for other common cancers. Checkpoint blockade (CTLA-4 or PD-1/L1 blockade) has become a major modality in the treatment of metastatic melanoma, and significantly prolongs survival of melanoma patients through inhibition of negative signaling in tumor-specific T-cells [3-7]. However, long-term survival and durable remission rates remain low, and new treatment options are needed to overcome low clinical response rate.

One promising avenue in animal models has been to add vaccination to anti-CTLA-4 therapy [9]. However, the addition of a gp100/IFA peptide vaccine did not enhance but instead slightly reduced the efficacy of anti-CTLA-4 monotherapy [3]. We recently reported that vaccine-intrinsic features of IFA-based peptide vaccines induce the sequestration, dysfunction and death of activated, tumor-specific T-cells at the inflamed vaccination site. Our recent preclinical data indicate that anti-CTLA-4-induced, melanoma-specific T-cells are also subject to this effect, resulting in lack of synergy and suggesting different, non-IFA-based vaccine approaches may be better candidates for combination with anti-CTLA-4 therapy [10].

Manipulating the tumor itself may be the best "vaccine"

Most vaccine studies have utilized non-mutated, differentiation antigens, such as gp100 [11], which have had real but modest clinical benefit. For example, we and others have shown that gp100 vaccination in combination with high dose IL-2 resulted in longer PFS in MM patients compared to those who received IL-2 alone [11]. However, recent evidence suggests that the most potent antigens may be mutated antigens, regarded as "foreign" by the immune system [12]. Anti-PD-1 therapy, for example, was effective in patients with MM and in patients with smoking-induced lung cancer, associated with large numbers of missense mutations due to UV rays and cigarettes, respectively. Robert Schreiber's group showed that mutated antigens were the most potent at eliciting murine tumor rejection [13, 14]. Finally, tumor-infiltrating lymphocytes (TIL) that upon adoptive transfer mediated MM rejection in patients also recognized mutated antigens [15]. Activation of potent tumor-specific T-cells has been shown to require ligation of the CD40 receptor on antigen presenting cells (APCs) [16]. Therefore, we hypothesize that using the tumor itself as a "vaccine site" by intratumoral injection of a CD40 agonistic molecule such as APX005M, will activate innate immunity and lead towards "immunizing" patients against their own unique mutated antigens. In preclinical models, we have tested the IT administration of the recombinant adenovirus encoding the dendritic cell-activating CD40L in mice and found CD8+ T-cell-mediated systemic activity against B16 melanoma. Importantly, IT administration of CD40L also augmented the activity of anti-CTLA-4 monotherapy. In this study we are testing the clinical efficacy, tolerability and immunological impact of IT administration of the CD40 agonistic mAb APX005M, in combination with anti-PD-1 mAb pembrolizumab in patients with MM.

We are utilizing the tumor itself as a "vaccine site" by activating innate immunity via IT administration of the CD40 agonistic mAb APX005M. Although others have attempted to enhance immunity at the tumor site, this study will be the first clinical trial of a CD40 agonistic monoclonal antibody in combination with pembrolizumab. Preclinical models suggest that combining a CD40 agonistic mAb with an anti-PD-1/PD-L1 mAb holds significant promise for inducing systemic anti-tumor immunity and tumor regression in patients with MM and other cancers [17, 18]

1.3 CD40 Activation Has a Crucial Role in Licensing DCs to Prime CD8+ T-cell Responses

CD40 is expressed on all APC such as DCs, macrophages and other cells. CD40 interaction with its ligand (CD40L) on DCs will result in their activation and maturation with subsequent upregulation of co-stimulatory molecules (including CD80, CD86 and CD70) [19] as well as of other TNF superfamily members, such as 4-1BBL, OX40L and GITR ligand. CD40 also induces the expression of pro-inflammatory cytokines, such as IL-1β, IL-6 and IL-12, by DCs [19] (Figure 1). Expression of CD40 on DCs is crucial for cytotoxic T lymphocyte (CTL) function and for CD8+ T-cell memory. CD40 activation on macrophages will lead to upregulation of TLR9 and an increase in tumoricidal activity, NO and TNF production.

Furthermore, CD40 may be expressed on tumor cells, and its ligation with its ligand induces re –expression of MHC I, rendering the cancer cell more immunogenic. In addition, CD40 activation may induce apoptosis of the cancer cells [20].

APX005M is a humanized IgG1 mAb. In vitro, APX005M binds to both human and cynomolgus monkey CD40 with high-affinity, triggering activation of B-cells, monocytes, and dendritic cells and stimulating cytokine release from both human and monkey lymphocytes and monocytes. APX005M does not bind to rodent CD40. In comparison with CP-870,893 and SGN-40, APX005M has both more potent CD40 agonistic effects and ADCP effector function. In vitro, APX005M exerts its anti-tumor activity via ADCP and induction of apoptosis in CD40+ tumor cells (e.g. CD40+ lymphoma xenograft models).

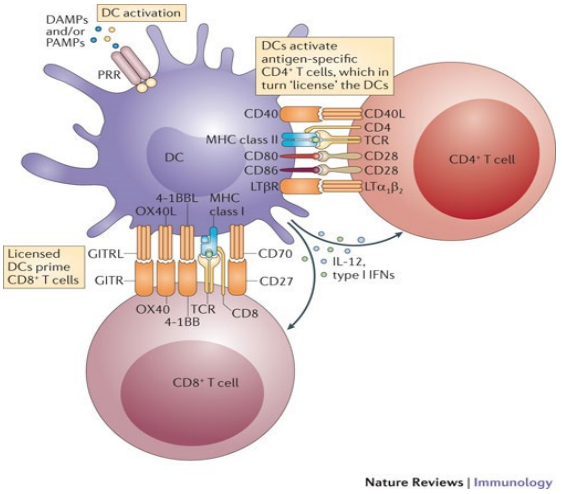


Figure 1: CD40 is at the interface between Innate and adaptive Immunity

Preclinical data suggests synergy between Intratumoral CD40 therapy and PD‑1 blockade.

Our preclinical studies show that IT injection of rAd.CD40L, a recombinant adenovirus expressing a stabilized version of the cognate CD40 ligand in mice harboring B16 tumors resulted in strong expansion of tumor infiltrating CD8 T-cells , in particular those with up-regulated PD-1+ as compared to empty vector, demonstrating the requirement for CD40L (Figure 2).

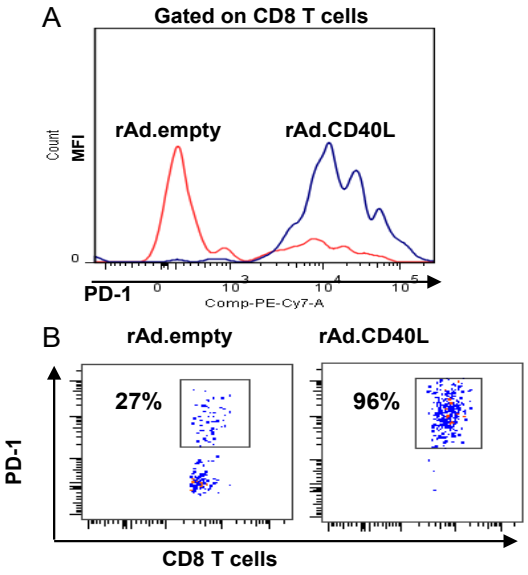


Figure 2: rAd.CD40L induces upregulation of PD-1 on tumor infilatrating CD8 T cells. (A&B) B.16F10 TILs were stained after 6 days of treatment for the presence of CD45 + CD8 + PD-1

We evaluated the effects of IT rAd.CD40L injections on non-injected, distant lesions. Importantly, Tumor-bearing mice receiving IT rAdCD40L in 1 tumor were able to suppress non-injected tumors on the opposite flank (Figure 3A), demonstrating the ability of rAd.CD40L to turn a locally injected tumor into a “vaccine site” capable of activating responses in distal lesions, and establishing a rationale to explore this approach in combination with systemic checkpoint blockades. In further experiments, we tested whether anti-PD-L1 would enhance the anti-melanoma activity of

rAd.CD40L. We found that rAd.CD40L strongly increased the therapeutic activity of anti-PD-L1 on rapidly progressing B16 melanoma tumors each agent dramatically reduced tumor growth and increased T-cell responses when combined with IT injection of rAd.CD40L (Figure 3B).

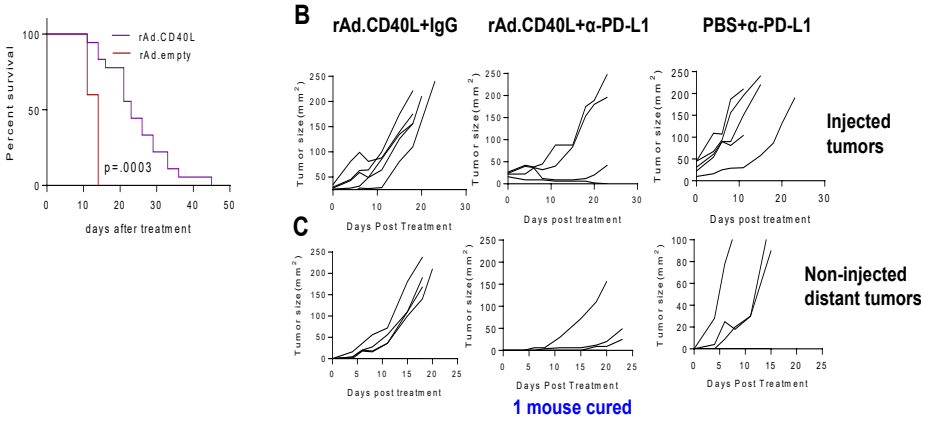


Figure 3: Intratumoral rAd.CD40L+α-PD-L1 suppress local and distant melanoma growth

(A) B16.F10 tumors were treated with rAd.CD40L or rAd empty. Overall mice survival is shown.

(B&C) Mice bearing subcutaneous B16.F10 tumors in left (B; 7-day tumor) and right (C; 3-day tumor) flanks were treated only in the left tumor on day 0, 4, and 11 with rAd.CD40L and received α-PD-L1 or IgG twice weekly tumor growth is plotted as individual mouse, n=5 or 20 mice in each group.

As expected, this anti-tumor activity of combination IT rAd.CD40L and anti-PD-L1 was dependent on CD8+ T-cells (data not shown). Thus, IT therapy with rAd.CD40L can enhance the systemic activity of checkpoint blockade therapy against distant, non-injected wild-type B16F10 melanomas. These results lay the groundwork to inform the design of our clinical studies testing a combination of PD-1 checkpoint blockade with IT and rAd.CD40L for MM.

IT CD40 activation plus systemic anti-CTLA-4 exert potent anti-melanoma effects.

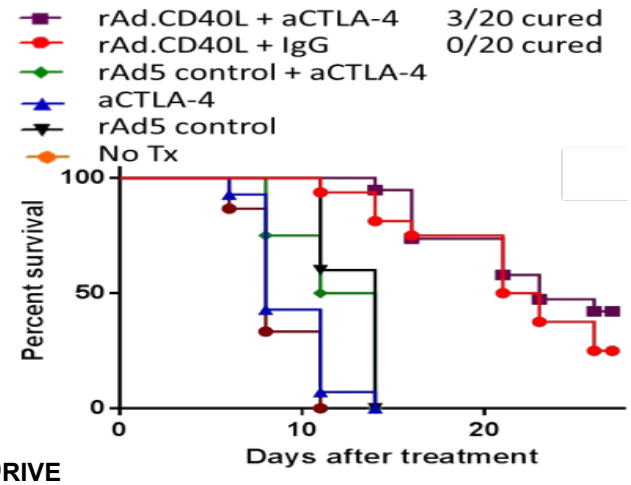
Our studies in defined peptide vaccine systems [9] and those of other groups show that the T-cell-inducing activity of TLR 7/8 agonists is greatly enhanced by concomitant provision of CD40 agonists. This combination potently activates antigen presenting cells to provide optimal priming of antigen-specific CD8+ T-cells [21].

We found robust activity of the combination of IT rAd.CD40L + systemic anti-CTLA-4 was very potent curing 3/20 mice of this very aggressive melanoma (Figure 4). The anti-tumor activity of combination IT rAd.CD40L and anti-CTLA-4 was dependent on CD8+ T-cells (data not shown).

Together, these results provide a strong rationale to test the combination of IT CD40 activation eventually with anti-CTLA-4 or anti-PD-1(checkpoint blockade) in patients with MM.

Figure 4: Therapeutic activity of rAd.CD40L + αCTLA-4 induces strong tumor suppression and complete, long-lasting tumor regression.

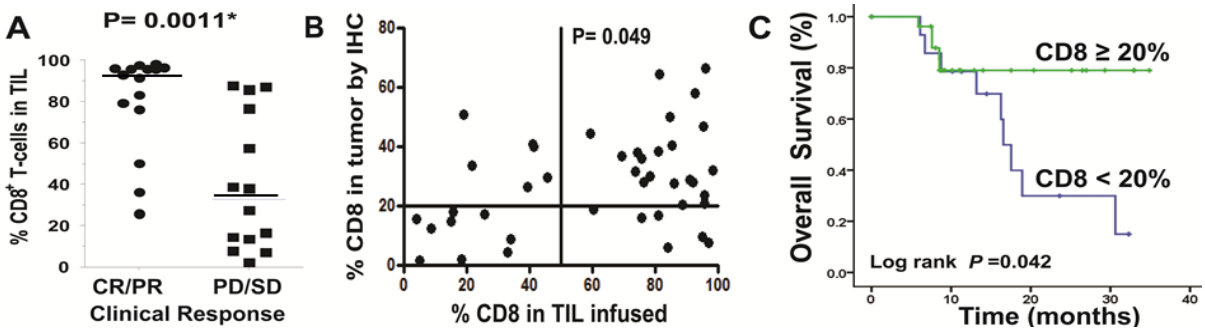
C57BL/6 mice bearing 7-day s.c. wild-type B16F10 melanomas received i.t. therapy with 1x1010 pfu rAd.CD40L or rAd5 control on day 0 and 7, and aCTLA-4 twice weekly. Tumor growth is plotted. Experiment still in progress; 3/20 mice cured in combination group.



1.4 CD8+ T-CELLS DRIVE RESPONSES TO TIL IN MELANOMA PATIENTS

Our adoptive T-cell transfer (ACT) study at MD Anderson Cancer Center [22] has demonstrated a strong positive correlation between the number of infused CD8+ TILs and clinical response in MM patients. In fact, a direct relationship was observed between the percentage of CD8+ T-cells found initially in the tumor biopsy and the ability to expand TIL to clinically significant numbers for infusion (data not shown) as well as the number of CD8+ TIL in the infusion product (Figure 5A,B). Patients with higher numbers of CD8+ T-cells in the initial tumor biopsy also had increased overall survival from the time of tumor harvest (Figure 5C), which is consistent with an earlier report [23].

Figure 5: Role of CD8+ T cells as predictive biomarkers in TIL ACT



(A) Percentage of CD8+ T cells within the infused TIL correlates with clinical response (N=31). (B) The frequency of CD8+ T cells in the tumor used to grow TIL is predictive of the frequency of CD8+ T cells in the expanded TIL product and (C) The frequency of CD8+ T cells in tumor is significantly associated with overall survival from the time of tumor harvest in TIL treated patients. (N=42 patients)

1.5 CLINICAL DATA OF CD40 AGONISTS

A few CD40 agonistic antibodies have been evaluated in human clinical trials. The majority of the clinical studies in cancer subjects with solid tumors have been conducted with the fully human IgG2 CD40 antibody CP-870,893. In a phase I clinical trial, CP-870,893 was well tolerated; the maximum tolerated dose (MTD) was found to be 0.2 mg/kg. The main toxicity of CP-870,893 was cytokine release syndrome (CRS) of mild to moderate severity. Anti-tumor activity was observed in several melanoma subjects treated with CP-870,893 [24].

A recent study indicated that CP-870,893 can also mediate an anti-tumor effect when combined with chemotherapy in subjects with metastatic pancreatic cancer [25]. Other CD40 agonistic antibodies that have been studied in human clinical trials are SGN-40 and ChiLob 7/4. SGN-40 is an IgG1 humanized anti-CD40 antibody, which has been tested predominantly in hematological malignancies as monotherapy or in combination with rituximab and chemotherapy [26, 27]. The major adverse effects of SGN-40 were anemia, pleural effusion, and thrombocytopenia [26].

ChiLob 7/4 is an IgG1 chimeric CD40 agonistic antibody that has been tested in a phase I clinical trial in subjects with solid tumors. The MTD of ChiLob 7/4 is 200 mg/weekly x4 and the major dose-limiting toxicity (DLT) was reversible liver enzyme elevation [28]. Although CP-870,893 has potent CD40 agonistic activities, it is an IgG2 antibody, and thus lacks antibody effector functions that constitute an important mechanism of action for CD40 antibody-mediated anti-tumor activities. SGN-40 is an IgG1 antibody, but it is a weak CD40 agonist. Due to its chimeric structure, ChiLob 7/4 might be immunogenic especially considering that the immune response is boosted by its CD40 agonistic effects.

1.6 APX005M

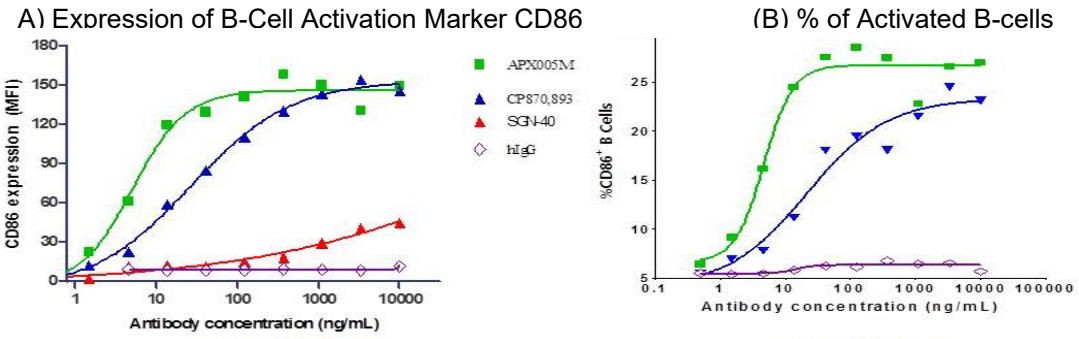
APX005M is a humanized IgG1 mAb. In vitro, APX005M binds to both human and cynomolgus monkey CD40 with high-affinity, triggering activation of B-cells, monocytes, and dendritic cells and stimulating cytokine release from both human and monkey lymphocytes and monocytes.

APX005M is an IgG1 humanized CD40 agonistic mAb with the S267E mutation at the Fc region. APX005M binds with high-affinity to human CD40 (Kd = 1.2 $\times 10^{-10}$ M) and monkey CD40 (Kd = 3.5×10^{-10} M), but does not cross-react with mouse or rat CD40. APX005M blocks the binding of CD40 to CD40L. In contrast, CP-870,893 and SGN-40 failed to block CD40 binding to CD40L suggesting that APX005M binds to an epitope that is different from the CP-870,893 and SGN-40 binding site(s).

Upon binding to CD40, APX005M activates the CD40 signaling pathway, leading to activation of APC, as seen in increased expression of CD80, CD83, CD86 and release of cytokines from human lymphocytes and monocytes. To benchmark APX005M with other CD40 agonistic antibodies, CP-870,893 and SGN-40 analogues were generated by Pyxis Oncology based on their published antibody sequences. In comparison with CP-870,893 and SGN-40 analogs, APX005M is the most potent CD40 agonist (Figure 6).

Upon binding to CD40+ tumor cells, APX005M is capable of inducing potent antibody effector functions such as ADCP (Figure 7A) and inducing tumor cell apoptosis (Figure 7B). APX005M does not appear to have a significant ADCP effect on DC (Figure 8).

Figure 6: APX005M is a Potent CD40 Agonistic Antibody

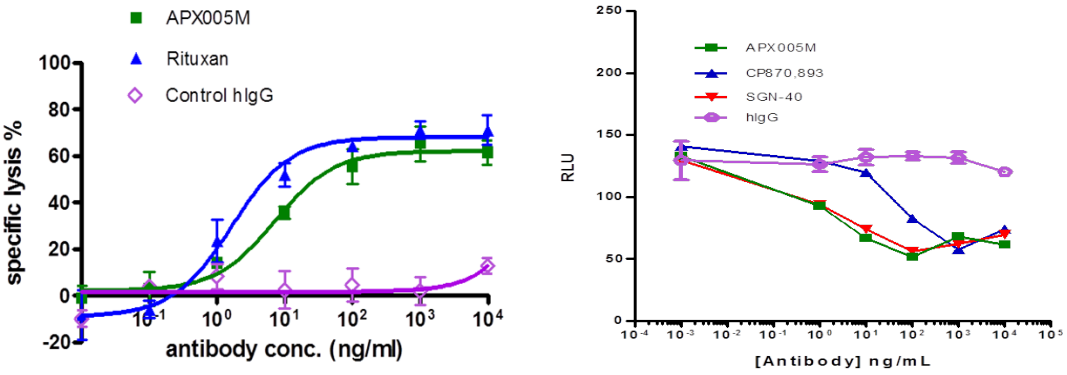


	EC50 (ng/ml)
APX005M	1.89
CP-870,893	12.01
SGN-40	1082.00

	EC50 (ng/ml)
APX005M	4.69
CP-870,893	21.62

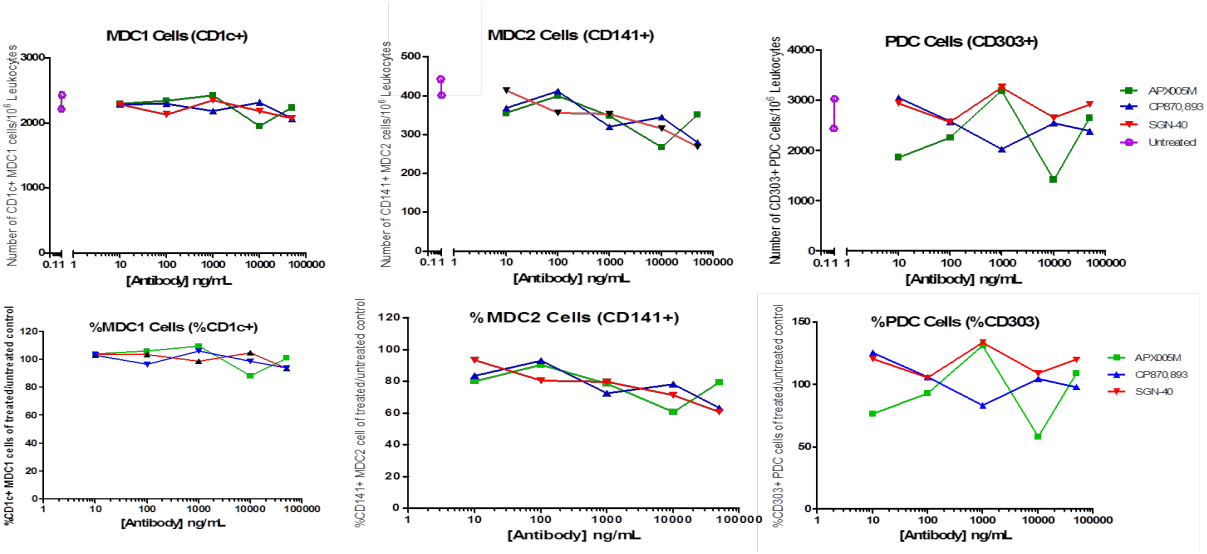
Human B cells were cultured with APX005M, CP-870,893 analog or SGN-40 analog for 48 hrs. B cell activation was quantified by measuring the expression of activation marker CD86 and % of CD86+ activated B cells.

Figure 7: Induction of Apoptosis and ADCP Effector Functions by APX005M on CD40+ Human Tumor Cells



Human lymphoma Ramos cells were co-cultured with human peripheral blood mononuclear cells (PBMCs) in the presence of APX005M, CP-870,893 analog or SGN-40 analog for 96 hrs and cell viability was measured using CellTiter-Glo (A). Ramos cells were cultured with antibodies for 72 hrs and cell viability was measured using CellTiter-Glo (B).

Figure 8: In Vitro Effect of APX on Human DCs



Human PBMC were cultured under conditions similar to those used in the ADCP assay with APX005M, CP-870,893 or SGN-40 for 4 hrs. Myeloid dendritic cells (MDCs) or plasmacytoid DCs (PDCs) were quantified using Miltenyi Biotech's Blood Dendritic Cell Enumeration Kit.

1.6.1 Preclinical Pharmacokinetics

Preclinical pharmacokinetics of APX005M was determined in the good laboratory practice (GLP) toxicology study using cynomolgus monkeys. Weekly intravenous administration of 5 doses of APX005M was well tolerated at 0.3, 3 and 30 mg/kg/dose. The PK properties of APX005M are typical of other mAbs and comprise low clearance (average range of 0.401-7.27 mL/h/kg), small volume of distribution (average range of 57-80.1 mL/kg) and long terminal half-life (average greater than 66 hours at 3 mg/kg and 30 mg/kg). Positive ADA titers were observed in all animals in the low dose group (0.3 mg/kg) but not in the high dose group (30 mg/kg). Based on these results, the no observed adverse effect level (NOAEL) was considered **30 mg/kg/dose.**

1.6.2 Animal Toxicology

The toxicology of APX005M was evaluated in 2 non-human primate (cynomolgus monkey) studies: a pilot single-dose non GLP study and a multi-dose GLP study.

The toxicology profile of APX005M appears distinct from that of the IgG2 CD40 agonist antibody CP-870,893, as no evidence of hepatotoxicity was evident in monkeys. The predominant effect seen with APX005M was a reduction in B-lymphocytes at doses > 3 mg/kg/dose consistent with that observed with CP-870,893 in prior studies and the pilot comparator toxicology study conducted with APX005M (23). These effects were prolonged and did not resolve over the course of the 28-day treatment-free recovery period. No evidence of any other clinical pathology effects was observed and APX005M was not associated with any anatomical pathology findings. Differences seen on selected flow cytometry and cytokine parameters in the pilot and definitive studies may be attributable to the larger number of animals evaluated in the definitive study or the timing of sample collection. With regard to the latter, samples were collected 24 hours postdose for flow cytometry and 10 minutes, 1, 2, 4, 8, and 24 hours for cytokine analyses in the single-dose pilot study. In the definitive study, sample collection for flow cytometry was conducted 48 hours post-administration (Days 1 and 15) and 24 and 48 hours post-administration on Day 29 (as well as during the recovery period) and cytokine sample collection performed 1, 4 and 24 hours following each dose. Positive

ADA titers were observed predominantly in low-dose (0.3 mg/kg/dose) animals. Exposure increased over the course of the GLP study in animals dosed > 3 mg/kg/dose.

The local tolerance was evaluated in the context of the repeat-dose GLP study in which gross and microscopic evaluation of the injection sites was conducted. No evidence of local irritation was observed in this study.

An in vitro hemolytic potential study was performed and consistent with the in vivo data in cynomolgus monkeys, no evidence of hemolytic activity was seen with the APX005M formulation.

In the tissue cross reactivity study, multiple cells/tissues exhibited specific binding to APX005M. In many cases the binding observed was cytoplasmic and thus, is of questionable clinical relevance. Selected human tissues exhibited specific binding (that was not cytoplasmic) that was not seen in monkeys. For these tissues it is possible that data derived from the monkey may not predict toxicity/effects in human. Based upon the monkey study results and those obtained with other similarly acting antibodies, hematological toxicities and/or cytokine-release related effects might occur at appropriate doses/exposures. These parameters will be monitored closely in the proposed clinical trial.

In this trial, unlike the monkey studies, APX005M, will be injected directly into the tumor lesions and not administered intravenously, which further minimize any potential toxicity, yet it is expected to be immunologically active dose.

1.6.3 Clinical Experience

Study APX005M-001 (NCT02482168) is an ongoing Phase 1, dose-escalation study of IV APX005M utilizing a modified 3+3 dose escalation design (the first 2 dose levels were single subject cohorts). As of 01 March 2016, 17 subjects have been enrolled in the Study APX005M-001 into 7 dose levels. Sixteen out of the 17 subjects enrolled have been withdrawn from study treatment, the majority of them due to progressive disease (by RECIST 1.1, or clinical progression). A summary of demographics and reasons for withdrawal of study treatment is provided in Table 1.

Table 1: Study APX005M-001 Enrollment by Dose Level, Sex, Race, Type of Cancer

	Study APX005M-001: Dose Levels and Dose (mg/kg)							
	DL1 (0.0001) N = 1	**DL2** (0.001) N =1	**DL3** (0.01) N =3	**DL4** (0.03) N = 3	**DL5** (0.1) N = 3	**DL6** (0.3) N = 3	**DL7** (1.0) N = 3	**All DLs N (%)** N = 17
Sex								
Male	0	0	1	2	1	0	1	5 (29.4)
Female	1	1	2	1	2	3	2	12 (70.6)
Race								
Asian					1			1 (5.9)
Black						1		1 (5.9)
White	1	1	3	3	2	2	3	15 (88.2)
Type of Cancer								
Anal				1				1 (5.9)
Colorectal			2	1	1	1	1	6 (35.3)
Endometrial	1				1			2 (11.8)
Hepatocellular					1			1 (5.9)
Melanoma						1		1 (5.9)
Pancreatic		1					2	3 (17.6)
SCC penis				1				1 (5.9)
Small Intestine			1					1 (5.9)
Unknown Primary						1		1 (5.9)
Reason For Withdrawal								
Disease progression	1	1	3	3	3	1	1	13 (76.5)
Transfer to hospice	-	-	-	-	-	1	-	1 (5.9)
Adverse Event	-	-	-	-	-	1	1	2 (11.8)

Data Cut-off 01 March 2016

Table 2: Study APX005M-001 Serious Adverse Events Regardless of Causality

Subj No.	System Organ Class Preferred Term	Event Grade	Study Drug Action Taken	SAE Criteria
3000-0001	GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Pyrexia	3	Dose Not Changed	Hospitalized
3000-0004	GASTROINTESTINAL DISORDERS			
	Intestinal obstruction*	3	Dose Not Changed	Hospitalized
3000-0004	INFECTIONS AND INFESTATIONS			
	Salmonellosis**	3	Dose Not Changed	Hospitalized
3000-0013	RENAL AND URINARY DISORDERS			
	Acute kidney injury	3	Dose Not Changed	Hospitalized
3000-0013	METABOLISM AND NUTRITION DISORDERS			
	Hyperuricaemia	4	Dose Not Changed	Hospitalized
9152-0012	GASTROINTESTINAL DISORDERS			
	Faecaloma	3	Dose Not Changed	Hospitalized
9152-0012	RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Pleural effusion	3	Dose Not Changed	Hospitalized
9182-0014	NERVOUS SYSTEM DISORDERS			
	Seizure	4	Drug Withdrawn	Hospitalized
9182-0016	IMMUNE SYSTEM DISORDERS			
	Cytokine release syndrome	4	Drug Withdrawn	Hospitalized

Drug Safety (Argus) Database extract 01 March 2016, reconciliation not complete with the clinical database
[*] Event Intestinal Obstruction is reported as “Abdominal Pain in the clinical database
[**] Event Salmonellosis is reported as “Salmonella bacteremia and enteritis: in the clinical database

The majority of adverse events reported were mild to moderate in severity (Table 3).

Table 3: Study APX005M-001 Number of Adverse Events by Grade and Number of Subjects by Maximum Grade of Adverse Event Experienced

	NCI CTCAE Grade					
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Not Graded
All AES	87 (71.3)	18 (14.8)	10 (8.2)	1 (0.8)	0	6 (4.9)
Number of Subjects*	4 (23.5)	7 (41.2)	4 (23.5)	1 (5.9)	0	

[*] 16/17 (94.%); one subject has no AEs reported

Most frequent AEs (reported in more than 2 subjects) independent of causality are presented in Table 4 and AEs considered related to APX005M are presented in Table 5.

Table 4: Study APX005M-001 Adverse Events in > 2 Subjects

Adverse Event **System Organ Class (OC)** **Preferred Term**	**All Grades**
GASTROINTESTINAL DISORDERS	
Abdominal distension	2 (11.8)
Abdominal pain	3 (17.6)
Abdominal pain upper	2 (11.8)
Constipation	2 (11.8)
Diarrhea	5 (29.4)
Gastroesophageal reflux disease	2 (11.8)
Nausea	4 (23.5)
Vomiting	4 (23.5)
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	
Asthenia	2 (11.8)
Chills	3 (17.6)
Edema peripheral	2 (11.8)
Fatigue	6 (35.3)
Pyrexia	4 (23.5)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	
Infusion related reaction	2 (11.8)
Weight decreased	2 (11.8)
METABOLISM AND NUTRITION DISORDERS	
Decreased appetite	5 (29.4)
Dehydration	2 (11.8)
Hyponatremia	3 (17.6)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	
Back pain	2 (11.8)
Muscle spasms	2 (11.8)
NERVOUS SYSTEM DISORDERS	
Dyspepsia	2 (11.8)
Headache	2 (11.8)

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS		
Cough	2	(11.8)
Dyspnea	4	(23.5)
Hypoxia	3	(17.6)
Pleural effusion	2	(11.8)
VASCULAR DISORDERS		
Hypertension	2	(11.8)

Table 5: Number (%) of Subjects in APX005M-001 with Adverse Events Considered Related to APX005M by System Organ Class and Grade

	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Any Grade
GASTROINTESTINAL DISORDERS						
Diarrhea	1 5.9%	1 5.9 %				2 11.8 %
Dry mouth	1 5.9%					1 5.9%
Vomiting	1 5.9%					1 5.9%
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS						
Chest discomfort	1 5.9%					1 5.9%
Chills	2 11.8 %					2 11.8 %
Fatigue	3 17.6 %					3 17.6 %
Malaise	1 5.9%					1 5.9%
Pyrexia	3 17.6 %					3 17.6 %
IMMUNE SYSTEM DISORDERS						
Cytokine release syndrome				1 5.9 %		1 5.9%
INJURY, POISONING AND PROCEDURAL COMPLICATIONS						
Infusion related reaction		1 5.9 %				1 5.9%
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS						
Myalgia	1 5.9%					1 5.9%
NERVOUS SYSTEM DISORDERS						
Dysgeusia	2 11.8 %					2 11.8 %
Headache	1 5.9%					1 5.9%
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS						
Hiccups	1 5.9%					1 5.9%
VASCULAR DISORDERS						
Flushing	1 5.9%					1 5.9%

Overall, IV administration of APX005M appears to be well tolerated at doses tested to date. AEs were consistent with this patient population and with the mechanism of action of a CD40 agonistic antibody. Preliminary pharmacodynamics data shows a dose dependent activation of antigen presenting cells in peripheral blood 24 hours after administration of APX005M.

1.6.4 Rationale for the Starting Dose

The proposed starting dose for APX005M of 0.1 mg (about 0.0014 mg/kg for a 70 kg person) was chosen based on the safety and biological activity observed in the ongoing APX005M single agent IV dose escalation Phase 1 study (APX005M-001). APX005M dosed up to 0.03 mg/kg was very well tolerated, with no ≥ Grade 2 drug related AEs or laboratory abnormalities.

The safety of APX005M is currently explored at the 1 mg/kg dose level, which is >700 times higher than the proposed starting dose for the intra-tumoral administration. The only severe (Grade 4)

toxicity observed to date with APX005M is CRS (at 1mg/kg dose level) which is non-overlapping with pembrolizumab toxicities.

In the IV study, the dose of 0.001 mg/kg of APX005M showed minimal biological effect as measured by expression of activation markers on antigen presenting cells in peripheral blood. The systemic exposure and biological activity is expected to be similar after IV and IT administration therefore the proposed stating dose of 0.1 mg is expected to be safe and provide minimal biological activity.

1.6.5 Packaging and Labeling

APX005M investigational product is supplied in 20 mL Type 1 clear glass vials labeled for intravenous injection. Each depyrogenated vial contains 16.9 ml (10 mg/mL) APX005M. APX005M is a sterile, clear to slightly opalescent, colorless to slightly yellow, preservative-free solution (pH 5.5) containing 25 mM sodium acetate, 248 mM trehalose, and 0.02% polysorbate 20 in water for injection (WFI) with a target fill volume of 16.9 mL per vial. Glass vials are plugged with Teflon coated rubber stoppers and sealed with aluminum seals. The 20 mL vials (16.9 mL/vial) are intended for single-use.

Additional APX005M details including labeling, storage, and preparation information for intratumoral administration are provided in the Pharmacy Manual. It should be noted that the Pharmacy Manual may be updated/revised as additional information becomes available.

1.6.6 Storage and Dispensing

The APX005M investigational product should be stored in a secure location with limited access under controlled temperature conditions of 2-8°C and in accordance with local regulations. Vials should be stored in their original folding carton to protect from light. If concerns regarding the quality or appearance of the investigational product arise, do not dispense the investigational product and contact the sponsor immediately.

The investigational product must be dispensed only from official study sites by authorized personnel according to local regulations. It is the responsibility of the investigator to ensure that investigational product is only dispensed to study subjects. APX005M must be administered to study subjects by qualified personnel.

Safety Precautions: When preparing investigational product wear laboratory coats and disposable protective gloves. Avoid contact with eyes, skin, and clothing. Protect from light and contamination.

1.6.7 Expired or Used APX005M

All expired or unused study drug will be reconciled and destroyed/disposed in accordance with the MDACC institutional policy for investigational drugs.

1.7 PEMBROLIZUMAB

Pembrolizumab (Keytruda™) is a highly-selective humanized mAb of IgG4/kappa isotype, designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. It was approved for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor since pembrolizumab has shown 26% clinical response in patients with ipilimumab refractory advanced melanoma [25]. Pembrolizumab will be purchased by M.D. Anderson Pharmacy from commercial sources and administered in accordance with manufacturer's guidance.

2 OBJECTIVES

2.1 PRIMARY OBJECTIVES

- To assess safety and tolerability of intratumoral APX005M given with systemic pembrolizumab and identify of the maximum tolerated dose/recommended phase 2 dose (MTD/RP2D) of the combination therapy in patients with metastatic melanoma (dose escalation phase)
- To assess overall response rate (ORR) at 12 weeks after intratumoral injection of APX005M in combination with systemic pembrolizumab (dose expansion phase)

2.2 SECONDARY OBJECTIVES

- To evaluate the immunological impact of IT APX005M given with systemic pembrolizumab by quantifying the tumor infiltrated CD8+T-cells (pre/post-therapy) both in injected and non-injected tumors
- To assess the overall safety and tolerability of IT APX005M given with systemic pembrolizumab in patients with metastatic melanoma (expansion phase)
- To evaluate anti-tumor immune responses and clinical efficacy of intratumoral injection of IT APX005M with systemic Pembrolizumab (dose escalation phase).

2.3 EXPLORATORY OBJECTIVES

- To explore potential associations between biomarker measures and anti-tumor activity
- To assess overall survival at 1 year and 2 years following the start of therapy.

3 MATERIALS AND METHODS

3.1 ELIGIBILITY CRITERIA

3.1.1 Inclusion Criteria

1. Be willing and able to provide written informed consent/assent for the trial.
2. Histologically or cytologically confirmed malignant melanoma arising from skin, or mucosal melanoma (i.e., ocular melanoma subjects are not eligible)
3. Measurable, unresectable stage III (in transit lesions) or stage IVA, IVB or IVC disease
4. At least 2 injectable lesions (amenable for direct injection or through the use of image guidance such ultrasound [US], CT or MRI) defined as any injectable cutaneous, subcutaneous, nodal, or visceral melanoma lesion ≥ 10 mm in longest diameter.
5. Age ≥ 18 year

6. ECOG performance status of 0 or 1

7. Total bilirubin less than or equal to 2.0 mg/dl, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dl

8. Platelet count greater than or equal to 100,000/mm^3

9. WBC > 3000/mm^3

10. ANC > 1500/mm^3

11. Hemoglobin >9 g/dL

12. Serum ALT and AST <3 the upper limit of normal (ULN); <5 ULN if there is liver involvement secondary to the tumor

13. Serum creatinine < 2.0 mg/dl

14. Seronegative for HIV antibody

15. Patients with a negative pregnancy test (urine or serum) must be documented within 14 days of screening for women of childbearing potential (WOCBP). A WOCBP has not undergone a hysterectomy or who has not been naturally postmenopausal for at least 12 consecutive months (i.e. who has not had menses at any time in the preceding 12 consecutive months).Unless surgically sterile by bilateral tubal ligation or vasectomy of partner(s), the patient agrees to continue to use a barrier method of contraception throughout the study and for 4 months after the last dose of APX005M or pembrolizumab such as: condom, diaphragm, hormonal, IUD, or sponge plus spermicide. Abstinence is an acceptable form of birth control.

3.1.2 Exclusion Criteria

1. Patients who have previously received pembrolizumab or PD-/L1 blockade therapy. Adjuvant IFN-α, is allowed if last dose was received at least 6 months of starting study treatment

2. Active autoimmune disease requiring disease-modifying therapy

3. Concurrent systemic steroid therapy higher than physiologic dose (>7.5 mg/day of prednisone or equivalent)

4. Any form of active primary or secondary immunodeficiency

5. History of hematologic malignancy

6. Active coagulopathy

7. History of New York Heart Association class 3-4 congestive heart failure or history myocardial infarction within 6 months of starting study treatment

8. History of arterial thrombosis within 3 months of starting study treatment

9. Patients with known symptomatic brain metastases requiring systematic corticosteroids. Patients with previous diagnosed brain metastases are eligible if they have completed their treatment and have recovered from acute effects of radiation therapy or surgery prior to the start of study medication, have discontinued corticosteroid treatment for their metastases for at least 2 weeks and are neurologically stable. Mild neurological deficits are allowed, if they do not interfere with the ability to judge the safety profile of APX005M.

10. Prior malignancy except the following: adequately treated basal cell or squamous cell skin cancer, in-situ cervical cancer, thyroid cancer (except anaplastic) or any cancer from which the patient has been disease-free for at least 2 years

11. Subjects who have received prior immune checkpoint inhibitors (e.g., anti-PD-1, anti-PD-L1), anti-CD40

12. Subjects that have received experimental vaccines or other immune therapies should be discussed with the Principal Investigator to confirm eligibility

13. Active known clinically serious infections (> Grade 2 NCI- CTCAE version 4.03)

14. Prior systemic therapy, radiation therapy, or surgery within the 28 days of starting study treatment. Palliative radiotherapy to a limited field or palliative cryoablation is allowed after consultation with the Principal Investigator, at any time during the study participation including screening

15. Women of child-bearing potential (WOCBP), women who are pregnant, or women who are nursing.

16. Known or underlying medical condition that, in the opinion of the investigator or sponsor, could make the administration of study drug hazardous to the subjects, or could adversely affect the ability of the subject to comply with or tolerate study procedures and/or study therapy, or confound the ability to interpret the tolerability of combined administration of APX005M and Pembrolizumab in treated subjects.

17. Has received a live vaccine within 30 days prior to the first dose of study drug. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette–Guérin (BCG), and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (eg, FluMist®) are live attenuated vaccines and are not allowed.

18. Has received a TB skin test within 14 days before the first dose of study drug.

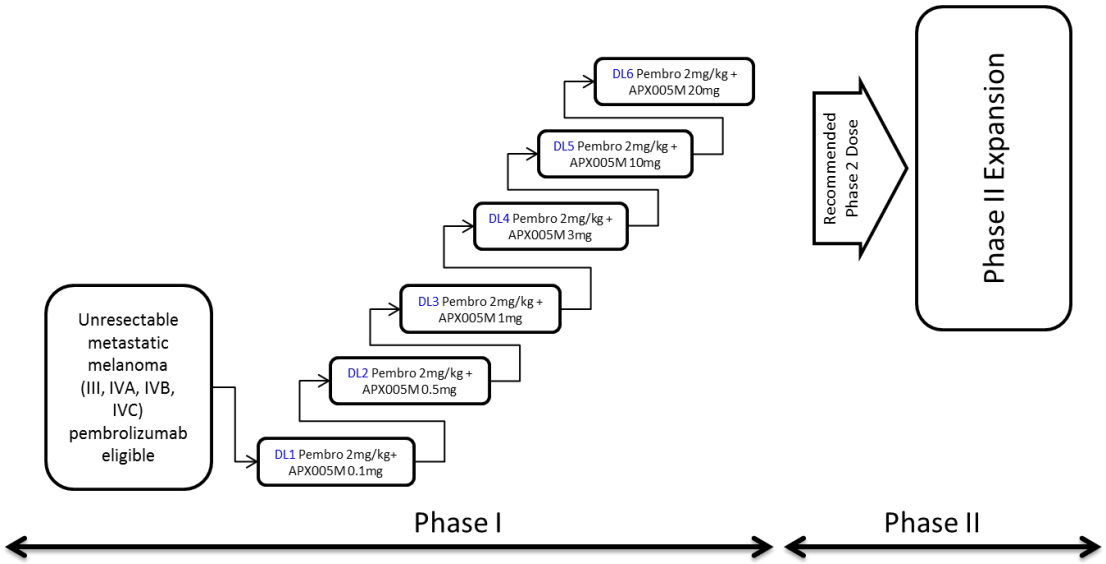
19. Other severe, acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or study treatment administration or that may interfere with the interpretation of study results and, in the judgment of the Investigator, would make the patient an inappropriate candidate for the study.

3.2 TREATMENT PLAN

3.2.1 Trial Design

This is a phase I/II, single-institution, clinical study which comprises a phase I dose escalation and a phase II expansion (Figure 9).

Figure 9: Scheme of the Trial Design



All subjects will receive standard-of-care pembrolizumab, every 3 weeks until progressive disease (PD), unacceptable toxicity or up to 2 years from study entry.

A cycle is defined as the 21 days (3 weeks) period starting with the administration of APX005M and pembrolizumab or pembrolizumab alone.

Both phases of the study will comprise of 3 periods:

- **Main Study:** is the 4 cycles (approximately 12 weeks) period during which subjects will receive up to 4 IT injections of APX005M along with IV pembrolizumab every 3 weeks (Figure 7) as long as they do not experience PD or unacceptable toxicity

- **Follow-up:** Subjects that did not experience PD or unacceptable toxicity may continue to receive IV pembrolizumab until PD, unacceptable toxicity or up to 2 years from study entry. Note that in the escalation phase of the study, patients receive 2 mg/Kg (max of 200 mg) of IV Pembrolizumab, and in the expansion phase of the study, there will be a flat dose of 200 mg given to patients.

- **Post Treatment Survival Follow-up:** All subjects that discontinue treatment for any reason will be followed-up for 2 years from study entry or until death, whichever occurs first

Intratumoral injection of APX005M: Injectable lesions (amenable for direct injection or through the use of image guidance such US, CT) may be defined as any injectable cutaneous, subcutaneous, nodal, or visceral melanoma lesion ≥ 10 mm in longest diameter.

(Measured by perpendicular diameter) Eligible subjects must have tumors with diameter perpendicular to longest diameter greater than 1cm to accommodate the anticipated injection volume:

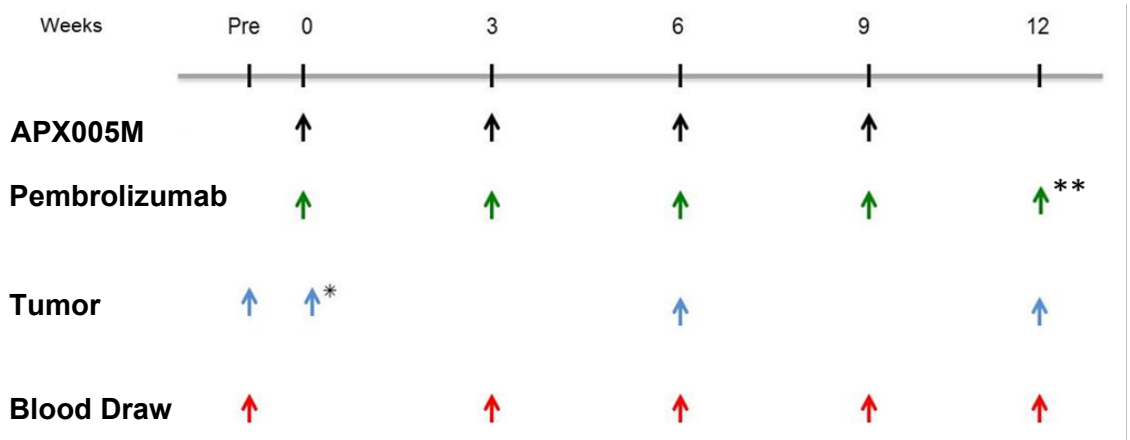
- Up to 1 ml will be injected into tumors 1cm to 1.5 cm
- Up to 2 ml into tumors 1.5 to 2.5 cm
- Up to 4 ml into tumors >2.5 cm

Pharmacokinetic models of monoclonal antibodies such as APX005M suggest an increase in maximum exposure with rapid/bolus administration of the drug therefore there is a risk for unknown or more severe side effects. To limit accidental systemic exposure to rapid administration of APX005M, splitting the total dose/volume into two injections, separated by an interval time of approximately 15 minutes (± 5 minutes), will be performed at each APX005M treatment timepoint, applicable to cohort 5 and the expansion phase.

Selection of injectable lesions should also take into accountability to obtain biopsies, accessibility, safety of the injection procedure (no encasing or in close proximity to crucial structure-carotid artery, jugular vein, trachea, etc.). The investigator together with the interventional radiologist will eventually determine if a tumor lesion is amenable for injection.

Due to tumor heterogeneity, and building on our experience from prior intratumoral therapeutic and ablative studies, soft tissue lesions are preferred as injectable and non-injectable target lesions. Also, based on our experience from prior studies, we anticipate that the large majority of all injections/biopsies will be image guided (US or CT), therefore they will be performed in the interventional radiology (IR) suite.

Figure 10: Main Study



✳: FNA to be done ~ 24 hrs. post injection

✳✳: Pembrolizumab administration continues q 3wks

The same tumor site will be injected for each of the 4 IT injections of APX005M.

All subjects will be monitored per institutional guidelines after each injection of APX005M and as clinically indicated thereafter. All subjects will be discharged from clinic after clinical evaluation. Subjects should have stable vital signs including: lack of orthostatic hypotension (systolic blood pressure >100 mmHg, or no lower than 10 mm from baseline) without IV hydration (no hydration for at least 2 hours prior to discharge), lack of hypoxia (oxygen saturation >90% without oxygen), temperature <38°C, heart rate <110 beats/min.

After discharge, all subjects should be monitored by a caregiver for 24 hours after the first 2 injections of APX005M and as clinically indicated thereafter.

Tumor biopsies and blood samples will be collected at time points indicated in Figure 10 from all subject enrolled in this study. Samples will be analyzed for T-cell specificity and phenotype, cytokine expression and antigen spreading using the assays as described in Appendix 1.

3.2.2 Phase 1 Dose Escalation

In the phase 1 dose escalation increasing dose levels (DL) of APX005M (Table 6) are combined with the approved dose and regimen of pembrolizumab.

The 21-day cycle starting with the first administration of IT APX005M and IV pembrolizumab represents the tolerability observation period.

A subject who meets all the study eligibility criteria, receives the entire planned dose of APX005M and pembrolizumab, and is assessed for the tolerability observation period (i.e., subject does not come off study for reasons other than toxicity) is considered evaluable. All subjects who do not meet these criteria will be replaced for the purpose of establishing the MTD.

Dose escalation of APX005M will be determined based on an accelerated 3+3 design using the following decision rules:

1) The 1st subject will enroll at DL1.

a) If this subject does not experience a Grade ≥ 2 adverse event that cannot be clearly attributable to extraneous causes (drug related adverse event [DRAE]), the dose will escalate to DL2.

b) If this subject experiences DRAE, 2 additional subjects will be enrolled at this dose level. If 0 of the 3 subjects experience a dose limiting toxicity (DLT), the dose will escalate to DL2. If any of the subjects experiences DLT the dose escalation will stop and no MTD will be established.

2) The 2nd or 4th subject will enroll at DL2.

a) If this subject does not experience DRAE, the dose will escalate to DL3.

b) If this subject experiences DRAE, 2 additional subjects will be enrolled at this dose level then follow rules in step 3.

3) If escalated, then enroll 3 subjects at the escalated dose.

a) If 0 of the 3 subjects experiences DLT, escalate to next dose level.

b) If 1 of the 3 subjects experiences DLT, then 3 additional subjects will be enrolled at the current dose level. If 0 of the additional 3 subjects experiences DLT, then escalate to next

dose level. If ≥ 1 of the additional 3 subjects experiences DLT, then de-escalate to previous dose level.

Repeat step 3 until all 5 dose levels have been explored. MTD will be defined as the highest dose level where ≤ 1 of 6 subjects experiences DLT.

In any of the multi-subject cohorts, the first 2 subjects must receive first dose of APX005M at least 72 hours apart.

In dose level 5 the total amount of APX005M could be administered to one tumor lesion with the dose being split equally between 2 syringes.

Table 6: Dose Levels (DL) and Number of Injection Sites

Dose Level (DL)	Injected tumor lesions	APX005M dose	Pembrolizumab	Minimum number of subjects
DL1 (Starting Dose)	1	0.1 mg	2 mg/kg	1
DL2	1	0.5 mg	2 mg/kg (max 200 mg)	1
DL3	1	1 mg	2 mg/kg (max 200 mg)	3
DL4	1	3 mg	2 mg/kg (max 200 mg)	3
DL5	(2 syringes)	10 mg	2 mg/kg (max 200 mg)	3 + 3
Expansion Cohort	1 (2 syringes)	10 mg	200 mg (flat dose)	20

There will be no intra-patient APX005M dose escalation above the assigned dose level at study entry.

All patients will receive standard-of-care pembrolizumab at 2 mg/kg every 3 weeks until PD, unacceptable toxicity or up to 2 years from study entry.

The recommended phase 2 dose (RP2D) of APX005M in combination with 200 mg IV pembrolizumab will be based on MTD and the overall safety and tolerability of IT APX005M in combination with IV pembrolizumab.

3.2.3 Phase 2 Expansion

In the Phase 2 Expansion portion of this study, 20 subjects will be enrolled to receive the RP2D of APX005M in combination with 200 mg flat dose of pembrolizumab.

The Expansion Phase dose of APX005M will be 10 mg.

3.3 CONCOMITANT TREATMENT

3.3.1 Premedication Prior to APX005M Administration and Guidance for Management of Cytokine Release Syndrome

Symptoms associated with CRS, including but not limited to chills, fever, rash, tachycardia, hypotension, rigor, and myalgias after IT administration of APX005M, are possible. In the ongoing Phase 1 dose escalation study APX005M-001 symptoms responded rapidly to supportive care in the outpatient setting.

If 2 or more Grade ≥ 2 CRS events are observed in two different subjects during the dose escalation, all subjects should be pre-medicated approximately 30 minutes before any administration of APX005M with a regimen containing (applicable to Cohort 5 and Expansion Phase):

- Oral H1 antagonist (e.g., loratadine 10 mg)
- Optional oral H2 antagonist (e.g., ranitidine 150−300 mg, cimetidine 300−800 mg, nizatidine 150−300 mg, and famotidine 20−40 mg)
- Oral non-steroidal anti-inflammatory drug (may comprise ibuprofen 400 mg or equivalent)
- Acetaminophen 650 mg

When the time between premedication and scheduled APX005M administration exceeds 4 hours, subjects may receive an additional course of premedication prior to APX005M administration.

CRS precautions should be observed during the administration of APX005M. Emergency agency agents including oxygen, oral and endotracheal airways, intubation equipment epinephrine, antihistamines, and corticosteroids should be available and used if required at the Investigator's discretion.

Subjects should be instructed that symptoms associated with CRS can occur at any time following the administration of the APX005M, and if such symptoms develop while they are at home, they should contact the Investigator and/or seek emergency medical care if appropriate.

If the subject experiences adverse events suggestive of infusion reaction/cytokine release syndrome, treat symptoms using the following guidance:

Suspected CRS-Related Toxicity	Recommended Treatment
Mild toxicity requiring symptomatic treatment only (e.g., fever, nausea, fatigue, headache, myalgia, malaise)	• Vigilant supportive care • Maintain adequate hydration • Antipyretics, non-steroidal anti-inflammatory drugs, antihistaminics, antiemetics, analgesics as needed • In case of mild symptoms persisting for > 24 hours assess for infections; empiric treatment of concurrent bacterial infections

<table>
<tr><td rowspan="2">Symptoms or clinical findings requiring and responding to moderate intervention, such as:

• O_2 requirement < 40%
• Hypotension responsive to fluids ± low dose of one vasopressor (e.g., < 50 mg/min phenylephrine)
• CTCAE Grade 2 organ toxicity</td><td>• No extensive co-morbidities</td><td>• All of the above
• Monitor cardiac and other organ functions closely</td></tr>
<tr><td>• Extensive co-morbidities
• Age ≥ 70 years</td><td>• All of the above
• Corticosteroids
• Consider tocilizumab</td></tr>
<tr><td colspan="2">Symptoms or clinical findings requiring aggressive intervention, such as:

• O_2 requirement ≥ 40%
• Hypotension requiring high dose or multiple vasopressors
• Ventilator support required
• CTCAE Grade ≥3 organ toxicity</td><td>• All of the above
• Corticosteroids
• Consider tocilizumab
• Discontinue APX005M</td></tr>
</table>

Steroids should not be used routinely to prevent or treat CRS as steroidal therapy may significantly impair the therapeutic benefit, however, these suggestions do not contraindicate the use of any medicine clinically needed under emergency circumstances including epinephrine, diphenhydramine, methylprednisolone or other steroids, nebulized albuterol, or any other medicine needed including additional narcotics to manage treatment-related symptoms as clinically indicated.

Ensure that subjects are well-hydrated prior to discharge.

- Consider administration of IV fluids

Instruct the subject to drink volume-increasing fluids (e.g. gatorade, broth) for the remainder of the infusion day and maintain an adequate oral fluid intake for the first 24 - 48 hours after APX005M administration

Consider prophylactic fever management for the first 24 hours (e.g. ibuprofen 400-600 mg every 8 hours, alternating with acetaminophen 1000 mg every 6 hours)

Consider withholding anti-hypertensive medications on the day of APX005M administration if in the opinion of the Investigator such action poses no risk to the subject.

3.3.2 Guidance for Management of Injection Site Reactions

Local injection site reactions are possible following IT injection of APX005M.

Only patients with cutaneous or subcutaneous lesions will be provided with a diary to collect information on the injection site(s) for 7 days after the IT injection (Appendix 3)

CTCAE Criteria for Injection Site Reactions

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Tenderness with or without associated symptoms (e.g., warmth, erythema, itching)	Pain; lipodystrophy; edema; phlebitis	Ulceration or necrosis; severe tissue damage; operative intervention indicated	Life-threatening consequences; urgent intervention indicated	Death

Note: Based on NCI CTCAE v.4.03. Definition of Injection Site Reaction: A disorder characterized by an intense adverse reaction (usually immunologic) developing at the site of an

Symptomatic treatment of injection site reactions should be consistent with the severity of the reactions as well as the institutional standards, and may include treatment with ice, acetaminophen, NSAIDS, antihistamines and narcotics. Due to their potentially immunosuppressive effects, the use of steroids should be avoided if clinically feasible. For severe reactions (Grade 3 or higher) inpatient admission with surgical consultation, and IV antibiotics will be required.

For treatment-related AEs </= Grade 2 that remain unresolved at the time of scheduled injection, no changes in dose or schedule are recommended. Grade 2 injection site reactions (despite optimal medical management) that remain unresolved at the time of scheduled reinjection should be managed as follows:

In the setting of Grade 2 local injection site reactions, an alternate easily accessible lesion, if available, should be considered for the injection of APX005M. If an alternate lesion cannot be identified, then injection of APX005M should be delayed until the event resolves to baseline or </= Grade 1. If the injection is delayed for more than one week, then the injection should be omitted and subsequent injections continued per original schedule. If 2 sequential scheduled injections are missed, the subject should be considered for withdrawal from the trial.

In the setting of second occurrence of persistent injection site reaction despite optimal medical management, APX005M should be permanently discontinued.

For treatment-related AEs=Grade 3, injection should be held until the toxicity resolves to Grade 1 or baseline. If resolution occurs within 14 days, then the investigator can consider continued dosing of APX005M at the next lowest dose level as long as the AE of concern was not considered life threatening, e.g. anaphylaxis, cytokine release syndrome, etc. If the toxicity does not resolve to </=Grade 1 or baseline within 14 days, then permanently discontinue APX005M.

For treatment related AEs >/= Grade 4, permanently discontinue APX005M.

3.3.3 Optional and Allowed Concomitant Medications

The following medications are optional and are allowed during the study:

- Anti-nausea and antiemetics (e.g., ondansetron, aprepitant, lorazepam) might be used for the prophylaxis or treatment of nausea and vomiting. National Comprehensive Cancer Network or institutional guidelines for treatment and prevention of nausea and vomiting should be followed

- Anti-diarrheals: For subjects developing Grade 1–2 diarrhea, loperamide (2 mg every 2 hours) is strongly recommended at the first onset of symptoms. For subjects with persistent diarrhea despite the use of loperamide, the use of octreotide is recommended. Other antidiarrheal agents may be used if necessary; a work-up for other etiologies is suggested for subjects who progress to Grade 3 or 4 diarrhea while taking loperamide

- Myeloid growth factors (e.g., granulocyte-colony stimulating factor) may be used if neutropenia occurs, in accordance with American Society of Clinical Oncology Guidelines,

but are not to be given prophylactically and should not be substituted for a required dose reduction

- Red blood cell transfusions, erythropoietic-stimulating agents, or platelet transfusions, if clinically indicated in accordance with institutional guidelines
- Bisphosphonates for bone metastases
- Treatment with non-conventional therapies (e.g., acupuncture), and vitamin/mineral supplements is acceptable provided that they do not interfere with treatment and the study endpoints
- Subjects may receive standard of care for any underlying illness or treatment emergent AEs
- Palliative radiotherapy during the study will be allowed for local pain control provided that:
    - In the opinion of the Investigator, the subject does not have progressive disease
    - The radiation field does not encompass the only target lesion (as defined by RECIST 1.1)
    - No more than 10% of the subject's bone marrow is irradiated
    - Note: Investigational product may be continued during palliative radiotherapy at the discretion of the Principal Investigator.

3.3.4 Prohibited and/or Restricted Therapies

Concurrent therapy with a marketed or investigational anti-cancer therapeutic, for either a palliative or therapeutic intent, is not allowed. Additionally, no alternative anti-cancer therapy (other than that administered in the study) or other investigational agents are allowed prior to treatment discontinuation. Herbal medicine for anticancer treatment should be stopped 1 week prior to 1st dose of investigational product.

Subjects taking narrow therapeutic index medications (such as warfarin, phenytoin, quinidine, carbamazepine, phenobarbital, cyclosporine, and digoxin) should be monitored proactively.

Concomitant treatment with systemic corticosteroids or other systemic immunosuppressive drugs is prohibited (excluding management of acute AEs). Please note that aromatase inhibitors, estrogen modulators, and adjuvant therapy is allowed (i.e. Arimidex).

TB (**tuberculosis)** Skin testing is prohibited 14 days prior to initial study treatment through 14 days post-treatment of APX005M.

Live vaccines within 30 days prior to the first dose of study treatment, while participating in the study, and 30 days post APX005M are prohibited. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (eg, FluMist®) are live attenuated vaccines and are not allowed.

3.4 CLINICAL PROCEDURES AND EVALUATION

3.4.1 Pretreatment Evaluation

At the screening visit, potential subjects will be assessed for study eligibility. All subjects must sign an informed consent form before enrollment and being registered in CORe/Prometheus. Consent will be obtained within 28 days of starting study treatment.

The following baseline assessments and procedures must be completed **within 28 days** of starting study treatment:

- Complete history, demographics, concurrent medication usage and physical examination including ECOG Performance Score, vital signs, height, weight, noting in detail the exact size and location of any lesions that exist will be performed
- Clinical Chemistry to include serum electrolytes, BUN, creatinine, glucose, albumin, alkaline phosphatase, ALT, AST, LDH, calcium, phosphorus, fractionated bilirubin, and magnesium
- CBC with differential and platelet count
- Coagulation: PT/PTT (prothrombin time/partial thromboplastin time)
- β-HCG pregnancy test (urine or serum) on all women of childbearing potential
- ECG
- HIV serology
- Thyroid function testing (TSH, Free-T_4)
- Baseline radiological studies to evaluate the status of disease (CT scans of chest, abdomen, pelvis; MRI/CT of brain) to evaluate the status of disease. Ultrasound or CT of area of in transit lesions is required.

The following pre-treatment assessments and procedures must be completed **within 7 days** of treatment initiation:

- β-HCG pregnancy test (urine or serum) on all women of childbearing potential
- Medical photography and measurements only obtained when Lesion A or Lesions A and B are in-transit lesions and/or cutaneous lesions. However, this will be case by case basis
- Baseline AE recording, physical exam and concurrent medication usage will be documented
- Baseline tumor biopsy (pretreatment) of the selected injection site (Injectable Non-Target or Lesion A) and non-injected site (Target or Lesion B)
- Blood sample draw (70 mL) for assays including but not limited to T-cell specificity and phenotype, cytokine expression

3.4.2 Main Study

During the Main Study Period subjects will receive up to 4 IT injections of APX005M along with IV pembrolizumab every 3 weeks and will be treated and evaluated as outlined below.

Cycle 1 (+2 Days)

Day 1:

- Physical Exam
- ECOG performance status
- Vital signs (includes height, weight, temperature, pulse, respiratory rate, systolic and diastolic blood pressure measurements) Please note that height is only taken at Screening.
- Record AEs and concomitant medication
- CBC with differential and platelet count
- PT/PTT
- Clinical Chemistry to include serum electrolytes, BUN, creatinine, glucose, albumin, alkaline phosphatase, ALT, AST, LDH, calcium, phosphorus, fractionated bilirubin, and magnesium
- β-HCG pregnancy test (urine or serum) on all women of childbearing potential
- Blood sample draw (70 mL) for assays including but not limited to T-cell specificity and phenotype, cytokine expression
- Medical photography and measurements only obtained when Lesion A or Lesions A and B are in-transit lesions and/or cutaneous lesions
- Tumor biopsy (harvest) of treated tumor (Lesion A) and non-treated tumor (Lesion B)
- First IT injection of APX005M

Day 2:

- Vital signs
- Record AEs and concomitant medication
- CBC with differential and platelet count
- One tumor biopsy of the APX005M injection site (Lesion A) to be collected at 24 hours (±7 hours) after first APX005M injection. This will occur prior to Pembrolizumab administration
- First dose of Pembrolizumab

Day 3

- Vital signs
- Record AEs and concomitant medication
- CBC with differential and platelet count
- Blood sample draw (70 mL) for assays including but not limited to T-cell specificity and phenotype, cytokine expression

Days 8 and 15 (± 1 day):

- Vital signs
- Record AEs and concomitant medication
- CBC with differential and platelet count
- Blood sample draw (70 mL) for assays including but not limited to T-cell specificity and phenotype, cytokine expression **(Day 8 ONLY)**
- Clinical Chemistry to include serum electrolytes, BUN, creatinine, glucose, albumin, alkaline phosphatase, ALT, AST, LDH, calcium, phosphorus, fractionated bilirubin, and magnesium

Cycle 2 (± 3 days)

Day 1:

- Verify criteria for treatment continuation (See Section 4.2)
- Physical Exam
- ECOG performance status
- Vital signs and weight
- Record AEs and concomitant medication
- CBC with differential and platelet count
- PT/PTT
- Clinical Chemistry to include serum electrolytes, BUN, creatinine, glucose, albumin, alkaline phosphatase, ALT, AST, LDH, calcium, phosphorus, fractionated bilirubin, and magnesium
- β-HCG pregnancy test (urine or serum) on all women of childbearing potential

- Blood sample draw (70 mL) for assays including but not limited to T-cell specificity and phenotype, cytokine expression
- IT injection of APX005M

Day 2:

- Vital signs
- IV pembrolizumab

Days 8 and 15 (± 1 day):

- Vital signs and weight
- Record AEs and concomitant medication
- CBC with differential and platelet count
- Clinical Chemistry to include serum electrolytes, BUN, creatinine, glucose, albumin, alkaline phosphatase, ALT, AST, LDH, calcium, phosphorus, fractionated bilirubin, and magnesium

Cycle 3 (± 3 days)

Day 1:

- Verify criteria for treatment continuation (See Section 4.2)
- Physical Exam
- ECOG performance status
- Vital signs and weight
- Record AEs and concomitant medication
- CBC with differential and platelet count
- PT/PTT
- Clinical Chemistry to include serum electrolytes, BUN, creatinine, glucose, albumin, alkaline phosphatase, ALT, AST, LDH, calcium, phosphorus, ~~and~~ fractionated bilirubin, and magnesium
- Thyroid Function Test (TSH and Free T4)
- β-HCG pregnancy test (urine or serum) on all women of childbearing potential
- Blood sample draw (70 mL) for assays including but not limited to T-cell specificity and phenotype, cytokine expression
- Tumor biopsy (harvest) of treated tumor (Lesion A) and non-treated tumor (Lesion B)

- IT injection of APX005M
- Medical photography and measurements only obtained when Lesion A or Lesions A and B are in-transit lesions and/or cutaneous lesions, only case by case basis

Day 2:

- Vital signs and weight
- IV pembrolizumab

Cycle 4: (± 3 days)

Day 1:

- Verify criteria for treatment continuation (See Section 4.2)
- Physical Exam
- ECOG performance status
- Vital signs and Weight
- Record AEs and concomitant medication
- CBC with differential and platelet count
- PT/PTT
- Clinical Chemistry to include serum electrolytes, BUN, creatinine, glucose, albumin, alkaline phosphatase, ALT, AST, LDH, calcium, phosphorus, fractionated bilirubin, and magnesium
- β-HCG pregnancy test (urine or serum) on all women of childbearing potential
- Blood sample draw (70 mL) for assays including but not limited to T-cell specificity and phenotype, cytokine expression
- IT injection of APX005M

Day 2:

- Vital Signs and Weight
- IV pembrolizumab

3.4.3 Follow-Up

Day 1 of Cycles 5-8 (± 3 days):

- Verify criteria for treatment continuation. See Section 4.2.
- Physical Exam
- ECOG performance status
- Vital signs and Weight
- Record AEs and concomitant medication
- CBC with differential and platelet count
- Clinical Chemistry to include serum electrolytes, BUN, creatinine, glucose, albumin, alkaline phosphatase, ALT, AST, LDH, calcium, phosphorus, fractionated bilirubin, and magnesium
- β-HCG pregnancy test (urine or serum) on all women of childbearing potential
- IV pembrolizumab

Day 1 of Cycle 5 and Cycle 8 (± 3 days):

- Radiological studies to evaluate the status of disease (CT scans of chest, abdomen, pelvis; (MRI/CT of the brain) to evaluate the status of disease. Ultrasound or CT of area of in transit lesions is required.

  NOTE: Radiological studies will be performed every 12 weeks after Cycle 8 until 2 years from patient's protocol entry date.

- Medical photography and measurements only obtained when Lesion A or Lesions A and B are in-transit lesions and/or cutaneous lesions, only case by case basis
- Blood sample draw (70 mL) for assays including but not limited to T-cell specificity and phenotype, cytokine expression
- Thyroid function testing (TSH and Free T4) and every 12 weeks after cycle 8 until 2 years from patient's protocol entry date.
- PT/PTT (Cycle 5 only)
- Mandatory tumor biopsy (harvest) of treated tumor (Lesion A) and if possible non-treated tumor (Lesion B) prior to Pembrolizumab infusion (Cycle 5 only). Biopsy is optional at progression of disease

Off treatment: is at time of documented progression as per RECIST 1.1, clinical progression, unacceptable toxicity, withdrawal of consent or significant noncompliance with the protocol. Off treatment procedures will include:

- Physical Exam
- ECOG performance status
- Vital signs and weight
- Record AEs and concomitant medication
- CBC with differential and platelet count
- Clinical Chemistry to include serum electrolytes, BUN, creatinine, glucose, albumin, alkaline phosphatase, ALT, AST, LDH, calcium, phosphorus, fractionated bilirubin, and magnesium
- Blood sample draw (70 mL) for assays including but not limited to T-cell specificity and phenotype, cytokine expression

3.4.4 Post Treatment Follow-Up

Subjects that have completed the Main Study and the Follow-up (pembrolizumab treatment per standard of care), will be followed every 12 weeks per the discretion of the treating physician until progression or for up to 2 years. If the patients wish to seek care at an outside institution after the completion of the Main study, they will be contacted every 12 weeks for up to 2 years from study entry to assess status of disease. Please note that these patients will be scanned at MDACC if possible.

3.4.5 Correlative Studies

3.4.5.1 Immunological Evaluations

Immunologic evaluations will be processed through M.D. Anderson's Immunotherapy Platform, which is a core processing facility within the Institution designed to help clinical researchers obtain immunologic expertise, guidance and immune monitoring analysis on patient blood and tumor samples. The Immunotherapy Platform provides the following services: (1) Isolation and cryopreservation of serum and peripheral blood mononuclear cells from sample (2) Data entry into Visual Specimen Manager (3) Sample transfer to liquid nitrogen for long-term storage (4) multiple cytokine analysis using serum (ELISA, Luminex) to measure antigen-specific cytokine production (5) Immunophenotyping with flow cytometry (6)The assessment of TCR Vß CDR3 clonal diversity will be done using ImmunoSeq™ (Adaptive Biotechnologies, Seattle, WA). Please see details in Appendix 1.

To evaluate the baseline tumor microenvironment, we will obtain biopsies from 2 tumors (A-Injected and B-not injected) before treatment if patients have ≥2 lesions. Then tumor A will be injected with APX005M, while tumor B will be left uninjected. After 6 weeks of treatment, tumors A and B will be biopsied again for analysis, TIL generation, and tumor cell line generation. One injected tumor will be harvested 24 hours after the 3rd dosing of APX005M + pembrolizumab, and when feasible one non-injected tumor will be harvested at the same time for TIL expansion. An optional biopsy will be obtained in week 12 to assess late changes to the immune microenvironment. One quarter of each biopsy will be evaluated using IHC, one quarter will be used for TIL growth, and DNA and RNA will be isolated from the remaining half. Since infiltration of tumors by CD8+ T-cells is a primary endpoint of this trial, this will be quantified by IHC. DNA isolated from treated and untreated tumors

resected for TIL expansion will be used in a new method called “QuanTILfy” to quantify T-cells in the tumor. Briefly, this assay will analyze matched 20 micron tumor FFPE tissue sections from untreated and treated tumor harvests used to grow TILs. DNA samples isolated from at least two FFPE tumor sections will used to assess TCR Vß CDR3 clonal diversity using ImmunoSeq™ (Adaptive Biotechnologies, Seattle, WA). If patients have only one injectable lesion, we will obtain FNA instead of punch biopsy from the lesion.

3.4.5.2 Biopsies

If possible, two areas will be biopsied during the course of the study including APX005M injected and non-injected sites. The first biopsy will be taken as a base line at any time after enrollment onto the trial, but prior to the first injection. Tumor biopsy of an injected site will be taken approximately 24 hours after initial injection to allow for analysis early and transient changes occurs on different immune cells in particular DCs. At week 9, before the 3rd injection, biopsy of both injected and non-injected sites will be taken. Tumor biopsies may be taken using any biopsy method to optimize tumor yield using standard sterile techniques. We anticipate most biopsies, patients will undergo biopsy (excisional, punch, or 4-6 passes by imaging-guided of both tumors (A-injected and B-non injected) before treatment. At least 1 injected and 1 non-injected tumor/lesion must not be biopsied to allow for preservation of measureable disease.

Blood and tissue specimens collected in the course of this research project may be banked and provided in the future to investigators with IRB approved research protocols.

4 EVALUATION OF TOXICITY

This study will utilize the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.03 for toxicity reporting. All appropriate treatment areas should have access to a copy of the NCI-CTCAE version 4.03.

4.1 DOSE LIMITING TOXICITY

DLT is defined as any of the following AEs attributed to APX005M (i.e., AEs that are not clearly attributable to extraneous causes), or AEs that are specific to pembrolizumab but are more severe that normally observed with pembrolizumab alone:

- Grade 4 neutropenia (ANC <500 cells/mm^3) for five or more consecutive days or Grade 3 or 4 neutropenia of any duration with sepsis or a fever greater than 38.5°C (oral). Grade 4 lymphopenia lasting less than 2 weeks will not be considered a DLT
- Grade 4 anemia
- Grade 4 thrombocytopenia (platelet count <25,000 cells/mm^3) or Grade ≥3 thrombocytopenia with signs or symptoms of bleeding or requiring platelet transfusion
- Grade ≥3 disseminated intravascular coagulation
- Grade ≥3 nausea, vomiting, or diarrhea despite the use of adequate/maximal medical intervention and/or prophylaxis (Grade 3 nausea, vomiting, or diarrhea that can be subsequently controlled in less than 72 hours [below Grade 3] with medical intervention or prophylaxis will not be considered a DLT)
- Grade ≥3 CRS
- Any other Grade ≥3 non-hematological and hematological toxicity except:

- Transient Grade 3 asymptomatic electrolyte abnormalities or transient Grade 3 elevation in liver function tests in subjects with liver metastasis have to be longer than 48 hours to be considered a DLT

- Grade 3 autoimmune thyroiditis that resolves to grade 1 or baseline within 28 days of onset

- Grade 3 AST/ALT/total bilirubin <8 x ULN if improves to Grade 1 within 7 days in patients who receive intrahepatic APX005M injections

- Delayed DLTs are drug-related adverse events that occur after Cycle 1. Delayed DLTs will not be used to determine the MTD for dose escalation. Delayed DLTs will be collected and evaluated by the Investigator on an ongoing basis. All AEs that meet criteria, as well as any >/= Grade 3 immune-related adverse events (regardless of attribution), must be reported to the IRB and Sponsor within 24 hours using the same rapid notification procedures that are used for serious AEs. Dosing of APX005M can be delayed for recovery from toxicity for up to 7 days.

- Delayed recovery from toxicity related to treatment with investigational product which delays scheduled retreatment for >3 weeks, or >1 week for cytokine release related complications.

4.2 CRITERIA FOR TREATMENT CONTINUATION

Subjects that do not experience PD may start a new cycle as scheduled if ANC >1,500/mm^3, platelets >100,000/mm^3, and disease or treatment-related non-hematologic toxicity has resolved to baseline or ≤ Grade 1. The following conditions are allowed to proceed:

- Grade 2 alopecia and Grade 2 fatigue, for which resolution is not required

- Grade 2 AST, ALT or total bilirubin for patients with liver metastasis who started treatment with Grade 2 AST/ALT/total bilirubin and the highest increases was <50% relative to baseline and lasted for less than 1 week

If a subject fails to meet criteria for retreatment then the treatment cycle should be delayed and the subject should be re-evaluated weekly. Any subject who fails to recover from a disease or treatment-related toxicity to baseline or ≤ Grade 1 (except alopecia and Grade 2 fatigue) within 12 weeks of scheduled retreatment should discontinue treatment. Failure to recover within 1 week of scheduled retreatment to baseline or ≤ Grade 1 of symptoms related to cytokine release should lead to APX005M discontinuation.

4.3 DOSE MODIFICATIONS

Management of suspected adverse drug reactions may require temporary interruption of both drugs and/or dose reduction of APX005M only. If a subject experiences several toxicities, the recommended dose adjustment should be based on the highest grade toxicity. All dose reductions for APX005M, if required, will follow the DLs defined in Table 7. If the APX005M dose needs to be reduced below DL1, treatment with APX005M will be discontinued. If more than 2 dose reductions are required for APX005M, subject will enter the Follow-up period (single agent pembrolizumab). There will be no dose modifications for pembrolizumab.

Table 7: Modified DLs for APX005M Following Qualifying AEs

DLs (mg)	DL1 (0.1)	DL2 (0.5)	DL3 (1)	DL4 (3)	DL5 (10)
First Dose Reduction	Discont.	DL1	DL2	DL3	DL4
Second Dose Reduction	NA	Discont.	DL1	DL2	DL3

4.3.1 Dose Modifications for Hematologic Toxicity

Dose adjustments are based on nadir blood counts since the preceding administration of investigational product. APX005M dose adjustments for hematologic toxicity during treatment are described in Table 8.

Table 8: APX005M Dose Modifications for Hematologic Toxicity

Drug related toxicity	**Action taken with APX005M**	**Action taken with Pemborlizumab**
Neutrophils (ANC) <500/mm^3 lasting ≥5 days	Dose reduction/Discontinue	Hold
Neutropenic fever (body temperature ≥38.5ºC (oral) and ANC <1000/mm^3)	Dose reduction/Hold	Hold
Platelets <25,000/mm^3	Dose reduction/Discontinue	Hold
Platelets <50,000/mm^3 with significant bleeding or requiring blood transfusion	Dose reduction/Discontinue	Hold
Grade ≥3 disseminated intravascular coagulation	Discontinue	Discontinue

4.3.2 Dose Modifications for Non-hematologic Toxicities

APX005M dose adjustments for non-hematologic toxicity during treatment are described in Table 9. All dose modifications should be made based on the worst preceding toxicity. For all toxicity ≤Grade 2, the current DL should be maintained.

Table 9: Dose Modifications for Non-hematologic Toxicity

Drug-related toxicity	Action taken with APX005M	Action taken with pembrolizumab
Grade 2 pneumonitis	Hold	Hold
Grade ≥2 colitis	Hold	Hold
Symptomatic hypophysitis	Hold	Hold
Grade 2 nephritis	Hold	Hold
Grade 3 hyperthyroidism	Hold	Hold
Nausea/vomiting ≥Grade 3 despite optimal medical treatment	Dose reduction/Discontinue	Hold
Diarrhea ≥Grade 3 despite optimal medical treatment	Dose reduction/Discontinue	Hold
CRS Grade 3 ≤7 days	Dose reduction	Hold
CRS Grade 3 >7 days	Discontinue	Hold
Grade ≥3 pneumonitis or nephritis	Discontinue	Discontinue
Other ≥3 Grade toxicities (except alopecia)	Hold, then adjust dose as medically indicated after discussion with Principal Investigator	Hold
Other Grade 4 event	Discontinue	Discontinue

Abbreviations: CRS = cytokine release syndrome.

4.4 DURATION OF TREATMENT

All subjects will receive up to 4 cycles of IV pembrolizumab in combination with IT APX005M followed by pembrolizumab single agent until documented disease progression, unacceptable toxicity, or up to 2 years from study entry.

If radiologic imaging shows PD, tumor assessment should be repeated ≥4 weeks later in order to confirm PD with the option of continuing treatment per below while awaiting radiologic confirmation of progression. If repeat imaging shows a reduction in the tumor burden compared to the initial scan demonstrating PD, treatment may be continued as per treatment calendar. If repeat imaging confirms progressive disease, subjects will be discontinued from study therapy. In determining whether or not the tumor burden has increased or decreased, Investigators should consider all target lesions as well as non-target lesions.

When feasible, subjects should not be discontinued until progression is confirmed; however, the decision to continue study treatment after the 1st evidence of disease progression is at the Investigator's discretion based on the clinical status of the subject as described in Table 10 below. Subjects that are deemed clinically unstable or who have biopsy proven new metastatic lesions are not required to have repeat imaging for confirmation of progressive disease.

Note: Treatment may be continued despite modified RECIST 1.1 defined progression if the subject is clinically stable and is considered to be deriving clinical benefit by the Investigator.

Clinical Stability is defined as:

- Absence of signs and symptoms (including worsening of laboratory values) indicating disease progression
- No decline in ECOG performance status
- Absence of rapid progression of disease
- Absence of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention

Table 10: Imaging and Treatment after 1st Radiologic Evidence of PD

	Clinically Stable		Clinically Unstable	
	Imaging	**Treatment**	**Imaging**	**Treatment**
1st radiologic evidence of PD	Repeat imaging at >4 weeks to confirm PD	May continue study Treatment at the Investigator's discretion while awaiting confirmatory scan	Repeat imaging at > 4 weeks to confirm PD if possible	Discontinue treatment
Repeat scan confirms PD	No additional imaging required	Discontinue treatment	No additional imaging required	N/A
Repeat scan shows SD, PR or CR	Continue regularly Scheduled imaging assessments every 6 weeks	Continue study treatment at the Investigator's discretion	Continue regularly scheduled imaging assessments every 6 weeks	May restart study treatment if condition has improved and/or clinically stable per Investigator's discretion
First scan is on Cycle 5. Second scan is on Cycle 8. Subsequent, Radiological Imaging will be performed every 12 weeks (+/- 7 days) thereafter.				

4.5 CRITERIA FOR REMOVAL FROM TREATMENT

Subjects will be taken off treatment for any of the following reasons:

- Decision to withdrawal of the consent for any reason

- Significant noncompliance (e.g.; failure to appear to more than 2 protocol specified procedures)
- Progression of disease
- Any toxicity requiring treatment discontinuation as outlined in the dose modification section of this protocol

5 CRITERIA FOR RESPONSE

Subjects will be evaluated for tumor response according to the RECIST 1.1 guidelines and also irRC (immune-related Response Criteria). Tumor measurements will be performed during the pretreatment evaluation, approximately every 6 weeks while subject remains in the main study and approximately every 9-12 weeks during follow-up up to 2 years from study entry.

For visible cutaneous or palpable subcutaneous tumors, target sites will be assessed by measurements of tumor size on clinical exam and photographically documented and placed in the patient's electronic chart.

For visceral or other subcutaneous or soft tissue metastases, radiologic evaluations in the form of CT scans of chest, abdomen, pelvis; MRI/CT of brain; ultrasound or CT of area of in transit lesions will be performed.

6 ADVERSE EVENTS

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Adverse event recording will occur up to 90 days after the last injection of APX005M.

6.1 SUSPECTED ADVERSE REACTION

Suspected adverse reaction (SAR) means any AE for which there is a reasonable possibility that the drug caused the AE. For IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the AE. SAR implies a lesser degree of certainty about causality than adverse reaction (AR), which means any AE caused by a drug.

SARs are the subset of all AEs for which there is a reasonable possibility that the drug caused the event.

6.2 ADVERSE REACTION

An AR means any AE caused by a drug. ARs are a subset of all SARs where there is reason to conclude that the drug caused the event.

6.3 LIFE-THREATENING

An AE or SAR is considered "life-threatening" if, in the view of either the Investigator or sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.

6.4 SERIOUS ADVERSE EVENT (SAE)

An AE or SAR is considered “serious” if, in the view of the investigator, it results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical events that may not result in death, but be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).
- Important medical events as defined above may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or IND Office.

6.5 UNEXPECTED ADVERSE EVENT

An AE or SAR is considered "unexpected" if it is not listed in the APX005M Investigator’s Brochure or is not listed at the specificity or severity that has been observed. "Unexpected," as used in this definition, also refers to AEs or SARs that are mentioned in the Investigator’s Brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation. Any condition, laboratory abnormality, or physical finding with an onset date prior to the subject signing the informed consent is considered to be pre-existing in nature and part of the subject’s medical history.

6.6 ADVERSE EVENT CLASSIFICATION

All AEs that are observed, elicited by the Investigator or appropriately qualified designee, or reported by the subject will be evaluated by the Investigator and recorded in the appropriate section of the case report form. The NCI-CTCAE v 4.03 should be used to describe the event.

6.6.1 Relationship to Study Drugs

The Investigator will assign attribution of the possible association of the event with use of the investigational drug, and this information will be entered into the case report form using the classification system listed below:

Related to Investigational Product

The event is suspected to be related if:

- There is a clinically plausible time sequence between the AE onset and administration of investigational product
- There is a biologically plausible mechanism for the investigational product to cause or contribute to the AE
- The event improves or diminishes upon temporary interruption of the investigational product without the initiation of any specific treatment for the event (dose delay) and/or recurs or worsens when resuming treatment after criteria for retreatment are met
- The AE cannot be reasonably attributed to concurrent or underlying illness, other drugs, or procedures

Unrelated to Investigational Product

- The event is not suspected to be related if:
- The AE is more likely to be explained by the subject's underlying disease, clinical state, concomitant medical, or study or non-study procedure
- The time occurrence of the AE is not reasonably related to administration of investigational product
- The event is not related to the investigational product.

6.6.2 Severity

The NCI-CTCAE v 4.03 should be used to assess the severity of AEs. For AEs not adequately addressed in the NCI-CTCAE version 4.03, Table 10 should be used.

Table 11: Toxicity Grading for AEs Not Covered in NCI-CTCAE (Version 4.03)

Severity	Description
GRADE 1 – Mild	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
GRADE 2 – Moderate	Minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of ADL
GRADE 3 – Severe	Medically significant but not immediately life threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
GRADE 4 – Life-threatening	Life-threatening consequences; urgent intervention indicated
GRADE 5 – Fatal	Death

Abbreviations: ADL = activities of daily living.

Abnormal laboratory findings should be reported as AEs only if they are clinically relevant.

6.7 COLLECTION AND REPORTING

All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in "The University of Texas M.D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Unanticipated Adverse Events for Drugs and Devices. Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).

All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to Pyxis Oncology (ds.apexigen@pyxisoncology.com), regardless of attribution (within 24 hours of knowledge of the event).

All life-threatening or fatal events, that are unexpected, and related to the study drug, must have a written report submitted within 24 hours (next working day) of knowledge of the event to the Safety Project Manager in the IND Office and Pyxis Oncology.

Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and MDACC IRB.

Serious adverse events of any causality will be captured from the time of informed consent, until 90 days after the last dose of APX005M, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.

Additionally, any serious adverse events that occur after the 90 day time period that are related to the APX005M and pembrolizumab combination must be reported to the IND Office and Pyxis Oncology (ds.apexigen@pyxisoncology.com). This may include the development of a secondary malignancy.

Reporting to FDA: Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

It is the responsibility of the Principal Investigator and the research teams to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines and Institutional Review Board policy**.**

The Principal Investigator will monitor the data and toxicities to identify trends. The principal investigator will be responsible for revising the protocol as needed to maintain safety. The M.D. Anderson IRB will review serious adverse events as they are submitted. Serious adverse events will be submitted to the FDA by the IND Safety Project Manager in the IND Office. The principal investigator will also review serious adverse events and evaluate trends. Whenever a trend is identified, the principal investigator will determine an appropriate follow up plan. The Principal Investigator or physician designee is responsible for verifying and providing source documentation for all adverse events and assigning the attribution for each event for all subjects enrolled on the trial.

7 STATISTICAL CONSIDERATIONS

7.1 DOSE ESCALATION

A minimum of 6 subjects and maximum of 30 subjects will be enrolled in the dose escalation phase of the study. The study design, dose levels, and decision rules for this phase are described in Section 3.2.2.

7.2 EXPANSION PHASE

An additional 20 patients will be enrolled at the MTD/RP2D dose level established in the dose escalation phase of the study. The objectives of the expansion phase are to assess ORR as well as to continue to evaluate safety of the treatment combination. Continuous monitoring of DLT will be assessed for all patients in the expansion phase, beginning with the first five patients. Assuming a prior beta distribution of (0.6, 1.4) which has a mean of 0.30 corresponding to a 30% targeted DLT rate, the expansion phase will terminate if the Pr ($\delta_t > 0.30 \mid$ data) > 0.80, where δ_t is the DLT rate attributable to the treatment combination. The decision rule for terminating for safety is presented in Table 13 and the operating characteristics (OCs) for this rule are presented in Table 14. The method used to produce the decision rule/stopping boundaries and OCs was designed by Thall, Simon, and Estey [29] and was implemented using the Multc Lean Desktop application (version 2.1) in the Biostatistics department. The monitoring rules will be assessed by the study team with assistance as necessary from the Department of Biostatistics at MD Anderson Cancer Center.

Table 12: Stopping Boundaries

Total number of patients	Stop if this many patients have toxicity:
1-4	Never stop with this many patients
5-6	3-6
7-8	4-8
9-11	5-11
12-14	6-14
15-17	7-17
18-19	8-19
20	Always stop at 20 patients

Table 13: Operating Characteristics

If the true DLT rate is…	Early Stopping Probability	Average Number of patients treated
0.1	0.018	19.7
0.2	0.137	18.3
0.3	0.399	15.2
0.4	0.704	11.4
0.5	0.908	8.3

7.2.1 Sample Size/Power for the Primary Efficacy Objective

The primary efficacy objective for this study is to assess ORR at 12 weeks of APX005M and pembrolizumab combined treatment. A sample size of 26 patients (6 from escalation phase + 20

from expansion phase) will have 75% power to detect an improvement from a null ORR of 33% to 55%, using a one group chi-square test and assuming a one-sided α–level of 5% (software: nQuery Advisor v7.0).

7.3 ENDPOINTS

7.3.1 Primary Endpoints

Safety and tolerability will be assessed by the rate of DLTs, incidence and severity of AEs and specific laboratory abnormalities graded according to NCI-CTCAE, v4.03 and by changes from baseline of vital signs and clinical laboratory results during and following study drug administration.

Categorical measures will be summarized using frequencies and percentages while continuous variables will be summarized using means, standard deviations, medians, minimums, and maximums. The primary efficacy endpoint (ORR) is defined as best overall response of complete response and partial response and will be estimated with 95% confidence intervals. The patients treated at the MTD/RP2D in the Phase I trial and those in the expansion cohort will be included in data analysis.

7.3.2 Secondary Endpoints

The correlation between change in CD8+ T-cell density of injected lesion and tumor shrinkage of non-injected lesion will be conducted based on Pearson's correlation coefficient and Spearman's rank correlation coefficient. Progression-free survival (PFS) will be defined from start of treatment to disease progression, death, or last evaluation assessment visit. Any patient who does not die or experience progression of disease by their last evaluation assessment visit will be censored. PFS will be estimated using the Kaplan-Meier method [31]. Cox proportional hazards regression models will be employed to assess the association between PFS and treatment and other clinical/demographic factors of interest. Duration of response (DOR) is defined as the time from the first evidence of confirmed PR or better to disease progression or death due to any cause.

7.3.3 Exploratory Endpoints

Associations between biomarker measures and anti-tumor activity will be assessed using Pearson's correlation coefficient and Spearman's rank correlation coefficient. Multiple testing adjustments using FDR will be considered. Overall survival (OS) will be defined from start of treatment to death or last evaluation assessment visit. Any patient who does not die by their last evaluation assessment visit will be censored. OS will be analyzed similar to PFS as described above.

7.3.4 Exploratory Studies

Associations between biomarker measures and anti-tumor activity will be assessed using Pearson's correlation coefficient and Spearman's rank correlation coefficient. Multiple testing adjustments using FDR will be considered. Overall survival (OS) will be defined from start of treatment to death or last evaluation assessment visit. Any patient who does not die by their last evaluation assessment visit will be censored. OS will be analyzed similar to PFS as described above.

7.3.5 Toxicity Summary Review

a. Phase I – Dose Escalation

A toxicity summary will be submitted to the IND Office Medical Monitor after the first evaluable patient completes 1 cycle of study therapy, and every evaluable patient thereafter. Accrual must be halted until summary is reviewed and study continuation is approved.

b. Phase II – Dose Expansion

An efficacy/toxicity summary will be submitted to the IND Office Medical Monitor when the first 5 evaluable patients complete 12 weeks of study therapy, and every 3 evaluable patients thereafter.

If there is no Grade 3 AE occurred with the first 5 evaluable patients of Dose Expansion, an efficacy/toxicity summary can be submitted every 5 evaluable patients thereafter.

8 DATA ENTRY AND PROTOCOL MANAGEMENT

For the purposes of this study at M. D. Anderson Cancer Center, the electronic data capture, Prometheus will be employed. All patients will be registered in CORe utilizing a two-turnstile registration before any study specific tests are performed. Concomitant medications will be captured in the medical record.

The principal investigator agrees to keep all information and results concerning the study confidential. The confidentiality obligation applies to all personnel involved with this clinical trial. The Investigator must ensure that each participant's anonymity will be maintained in accordance with applicable laws. The principal investigator should keep a separate log of ID numbers, names and addresses. Documents that contain the names associated with these ID numbers (e.g., written consent/assent forms), should be maintained by the Investigator in strict confidence except to the extent necessary to allow auditing by regulatory authorities, auditing or monitoring by the IRB.

The Principal Investigator shall obtain all such permissions and authorizations as may be necessary or desirable to allow the collection and use of information protected under Federal privacy laws and State privacy laws, including permission/authorization for monitoring and analysis (including re-analysis in combination with results of other studies), for regulatory submission purposes and for applicable reporting (if any).

9 ADMINISTRATIVE PROCEDURES

9.1 CHANGES TO THE PROTOCOL

Any change or addition to this protocol requires a written protocol amendment that must be approved by the IND Office and the IRB. A copy of the written approval of the IRB must be received by the IND Office and the principal investigator before implementation of any changes. The IRB must review and approve all amendments to the protocol. This study will be monitored for compliance by the IND office.

9.2 ETHICS AND GOOD CLINICAL PRACTICE

This study must be carried out in compliance with the protocol and Good Clinical Practice, as described in:

- ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
- US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
- Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

- The investigator agrees, when signing the protocol, to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice

10 REFERENCES

1. American Cancer Society AC: Cancer Facts & Figures 2014.

2. Bradford PT, Freedman DM, Goldstein AM, Tucker MA: Increased risk of second primary cancers after a diagnosis of melanoma. Archives of dermatology 2010, 146(3):265-272.

3. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC et al: Improved survival with ipilimumab in patients with metastatic melanoma. The New England journal of medicine 2010, 363(8):711-723.

4. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, Segal NH, Ariyan CE, Gordon RA, Reed K et al: Nivolumab plus ipilimumab in advanced melanoma. The New England journal of medicine 2013, 369(2):122-133.

5. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K et al: Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. The New England journal of medicine 2012, 366(26):2455-2465.

6. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB et al: Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. The New England journal of medicine 2012, 366(26):2443-2454.

7. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, Wolchok JD, Hersey P, Joseph RW, Weber JS et al: Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. The New England journal of medicine 2013, 369(2):134-144.

8. Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, Byrd DR, Buzaid AC, Cochran AJ, Coit DG, Ding S et al: Final version of 2009 AJCC melanoma staging and classification. Journal of clinical oncology 2009, 27(36):6199-6206.

9. Wada S, Jackson CM, Yoshimura K, Yen HR, Getnet D, Harris TJ, Goldberg MV, Bruno TC, Grosso JF, Durham N et al: Sequencing CTLA-4 blockade with cell-based immunotherapy for prostate cancer. Journal of translational medicine 2013, 11:89.

10. Hailemichael Y, Dai Z, Jaffarzad N, Ye Y, Medina MA, Huang XF, Dorta-Estremera SM, Greeley NR, Nitti G, Peng W et al: Persistent antigen at vaccination sites induces tumor-specific CD8(+) T-cell sequestration, dysfunction and deletion. Nature medicine 2013, 19(4):465-472.

11. Schwartzentruber DJ, Lawson DH, Richards JM, Conry RM, Miller DM, Treisman J, Gailani F, Riley L, Conlon K, Pockaj B et al: gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. The New England journal of medicine 2011, 364(22):2119-2127.

12. Overwijk WW, Wang E, Marincola FM, Rammensee HG, Restifo NP: Mining the mutanome: developing highly personalized Immunotherapies based on mutational analysis of tumors. Journal for immunotherapy of cancer 2013, 1:11.

13. Matsushita H, Vesely MD, Koboldt DC, Rickert CG, Uppaluri R, Magrini VJ, Arthur CD, White JM, Chen YS, Shea LK et al: Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. Nature 2012, 482(7385):400-404.

14. Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, Ivanova Y, Hundal J, Arthur CD, Krebber WJ et al: Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. Nature 2014, 515(7528):577-581.

15. Robbins PF, Lu YC, El-Gamil M, Li YF, Gross C, Gartner J, Lin JC, Teer JK, Cliften P, Tycksen E et al: Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T-cells. Nature medicine 2013, 19(6):747-752.

16. Sotomayor EM, Borrello I, Tubb E, Rattis FM, Bien H, Lu Z, Fein S, Schoenberger S, Levitsky HI: Conversion of tumor-specific CD4+ T-cell tolerance to T-cell priming through in vivo ligation of CD40. Nature medicine 1999, 5(7):780-787.

17. Winograd R, Byrne KT, Evans RA, Odorozzi PM, Meyer ARL, Bajor DL, Clendenin C, Stanger BZ, Furth EE, Wherry EJ, Vonderheide RH. Induction of T-cell immunity overcomes complete resistance to PD-1 and CTLA-4 blockade and improves survival in pancreatic carcinoma. Cancer Immunol Res. 2015;3(4):399–411.

18. Zippelius A, Schreiner J, Herzig P, Muller P. Induced PD-L1 expression mediates acquired resistance to agonistic anti-CD40 treatment. Cancer Immunol Res. 2015;3(3):236–244.

19. Summers deLuca L, Gommerman JL: Fine-tuning of dendritic cell biology by the TNF superfamily. Nature reviews Immunology 2012, 12(5):339-351.

20. Khong A, Nelson DJ, Nowak AK, Lake RA, Robinson BW: The use of agonistic anti-CD40 therapy in treatments for cancer. International reviews of immunology 2012, 31(4):246-266.

21. Ahonen CL, Doxsee CL, McGurran SM, Riter TR, Wade WF, Barth RJ, Vasilakos JP, Noelle RJ, Kedl RM: Combined TLR and CD40 triggering induces potent CD8+ T-cell expansion with variable dependence on type I IFN. The Journal of experimental medicine 2004, 199(6):775-784.

22. Radvanyi LG, Bernatchez C, Zhang M, Fox PS, Miller P, Chacon J, Wu R, Lizee G, Mahoney S, Alvarado G et al: Specific lymphocyte subsets predict response to adoptive cell therapy using expanded autologous tumor-infiltrating lymphocytes in metastatic melanoma patients. Clinical cancer research 2012, 18(24):6758-6770.

23. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, Tosolini M, Camus M, Berger A, Wind P et al: Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006, 313(5795):1960-1964.

24. Vonderheide RH, Flaherty KT, Khalil M, Stumacher MS, Bajor DL, Hutnick NA, Sullivan P, Mahany JJ, Gallagher M, Kramer A et al: Clinical activity and immune modulation in cancer patients treated with CP-870,893, a novel CD40 agonist monoclonal antibody. Journal of clinical oncology 2007, 25(7):876-883.

25. Beatty GL, Chiorean EG, Fishman MP, Saboury B, Teitelbaum UR, Sun W, Huhn RD, Song W, Li D, Sharp LL et al: CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. Science 2011, 331(6024):1612-1616.

26. Lewis TS, McCormick RS, Stone IJ, Emmerton K, Mbow B, Miyamoto J, Drachman JG, Grewal IS, Law CL: Proapoptotic signaling activity of the anti-CD40 monoclonal antibody dacetuzumab circumvents multiple oncogenic transformation events and chemosensitizes NHL cells. Leukemia 2011, 25(6):1007-1016.

27. Forero-Torres A, Bartlett N, Beaven A, Myint H, Nasta S, Northfelt DW, Whiting NC, Drachman JG, Lobuglio AF, Moskowitz CH: Pilot study of dacetuzumab in combination with rituximab and gemcitabine for relapsed or refractory diffuse large B-cell lymphoma. Leukemia & lymphoma 2013, 54(2):277-283.

28. Johnson PW, Challis R, Chowdhury F, Gao Y, Harvey M, Geldart T, Kerr P, Chan HT, Smith A, Steven NM, Edwards C, Ashton-Key M, Hodges E, Alison L Tutt AL, Ottensmeier CH, Glennie MJ, Williams AP. Clinical and biological effects of an agonist anti-CD40 antibody. A Cancer Research UK phase I study. Clinical cancer research 2015, 21(6):1321–8.

29. Thall PF, Simon RM, Estey EH: Bayesian sequential monitoring designs for single-arm clinical trials with multiple outcomes. Statistics in medicine 1995, 14(4):357-379.

31. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. Journal of the American Statistical Association 1958(53):457-481

11 APPENDICES

Appendix 1: Priority of Assays and Quantitative Parameters for Analysis

Tissue	End Point Type	Parameter	Modality	Assay	Quantitative Measurement	Priority
Tumor	Primary	CD8+ infiltration into tumor (% CD8 T cells).	Flow Cytometry	Specific antibodies for CD3/4/8 and use of a dump channel	Percentage/number of CD8 T cells normalized per infiltrating Lymphocytes (CD45+) and/or tumor weight	1
			IHC	Specific Antibodies	Number of CD8 T cells normalized per field	
	Secondary	T-cell specificity for de novo antigens*	Mutanome	Exome Sequencing and *in silico* identification of candidate peptiedes	Identification of tumor-specific mutations	2
	Secondary	T-cell specificity (both de novo mutated antigens and Melanoma Differentiation Antigens – MDAs)	1) Flow Cytometry using UV-Induced p*MHC peptide exchange technology[1] or Dextramers)	P*MHC peptide Exchange with mutanome/MDA identified peptides (or MDA Dextramers)	Percentage of antigen specific CD8+ T-cells which are positive for mutated peptide/MDA loaded-pMHC/Dextramers or induce IFN-γ production (ELISPOT)	3
			2) ELISPOT	ELISPOT with identified mutant/MDA peptides		
	Secondary	T-cell Infiltration	PCR	Quan TILfy	Enumeration of tumor-infiltrating T-cell numbers	4
	Secondary	Immune cell function	qRT-PCR	qRT-PCR for cytokine gene expression	Relative expression of cytokine genes compared to control (treated pt to untreated pt; treated tumor to untreated tumor)	5
	Secondary	T-cell clonal diversity	PCR	High-throughput Bβ CDR3 region sequencing technology	T-cell clone Tracking through Vβ CDR3 region sequencing	6
	Secondary	Immune cell phenotype	Flow Cytometry	Antibodies specific for NK cells, pDC/mDC and to characterize T cells: effector-memory panel and Treg (FoxP3)	Percentage of cells expressing markers	7

Appendix 2: Study Schedule of Events

	Screening		Treatment Period										Follow-Up [q]	Early Discontinuation or EOT
Cycle [a]	(≤28 Days)		1					2			3	4	5+	
Day	-28 to 0	-7 to 0	1 (+2 days	2	3	8 (±1 day)	15 (±1 day)	1 (±3 days)	8 (±1 day)	15 (±1 day)	1 (±3 days)	1 (±3 days)	1 (±3 days)	
Study Assessments														
Informed consent	x													
Demographic data	x													
Medical History [b]	x													
Physical Exam	x	x	x					x			x	x	x	x
ECOG Performance Status	x		x					x			x	x	x	x
Vital Signs, Height(for screening only) Weight [c]	x		x	x	x			x	x	x	x	x	x	x
Medications [d]	x		x	x	x	x	x	x	x	x	x	x	x	x
Adverse Events			x	x	x	x	x	x	x	x	x	x	x	x
Clinical Chemistry [e]	x		x			X	X	x	x	x	x	x	x	x
CBC with differential and platelet count	x		x	x	x	x	x	x	x	x	x	x	x	x
Coagulation (PT/PTT) (up to Cycle 5)	X		X					X			X	X	X	
β-HCG Pregnancy Test [f]	x	X						X			x	X	X	X
ECG	x													
HIV Serology	x													
Thyroid Function Testing [g]	x										x		x	
Blood Sample – for Assays [h]			x		x	x		x			x	x	x	✱
Blood Sample – Cytokines Measurement			x											
Radiological Studies [i]	X												x	
Medical Photography and Measurements [j]	X												x	
Tumor Biopsy (Pretreatment)([k]			X								X		x	X
Tumor Biopsy (Post APX005M) [l]				X										
APX005M IT Injection[r]			x					x			X	x		
Pembrolizumab IV [n]				x				x			x	x	x	
Treatment Continuation Verification [p]			x					x			X	X	X	

[a] Each cycle is 21 days
[b] A complete medical history must be completed. Additionally at the Screening Visit, in detail, the exact size and location of any lesions that exist must be noted.
[c] Vital signs include temperature, pulse, respiratory rate, systolic and diastolic blood pressure measurements. Height and weight are collected at the Screening Visit.
[d] Concurrent medications will be collected within 28 days of beginning treatment. Concomitant medication will be collected at the start of treatment through the end of study participation.
[e] Clinical Chemistry consists of: Serum electrolytes, BUN, creatinine, glucose, albumin, alkaline phosphatase, ALT, AST, LDH, calcium, phosphorus,fractionated bilirubin, and Magnesium.

	Screening		Treatment Period										Follow-Up [q]	Early Discontinuation or EOT
Cycle [a]	(≤28 Days)		1					2			3	4	5+	
Day	-28 to 0	-7 to 0	1 (+2 days	2	3	8 (±1 day)	15 (±1 day)	1 (±3 days)	8 (±1 day)	15 (±1 day)	1 (±3 days)	1 (±3 days)	1 (±3 days)	
Study Assessments														

[f] β-HCG Pregnancy test (urine or serum) will be conducted on all women of child-bearing potential before dosing of each cycle.. A negative pregnancy test must be confirmed at Screening and again with 7 days of the start of treatment.

[g] Thyroid Functioning Tests for this protocol include: TSH and Free-T_4 only. Thyroid function test will be done at Screening, C3, C5, C8 and then every 4 cycles

[h] 70mL blood sample draw for assays including, but not limited to T-cell specificity and phenotype, cytokine expression, will be collected C1D1, C1D3, C1D8, C2D1, C3D1, C4D1, C5D1, and C8D1

[i] Radiological studies to evaluate the status of disease (CT scans of the chest, abdomen, pelvis with/without contrast; MRI/CT of brain, Ultrasound or CT of area of in-transit lesions) is required at baseline and before C5 and C8 (+/- 7 days) and every 12 weeks (+/- 7 days) thereafter.

[j] Medical photography and measurements only obtained when Lesion A or Lesions A and B are in-transit lesions and/or cutaneous lesions. Please note that intransit lesions will be captured by scans. Medical photography and measurements will be taken on a case by case basis.

[k] Pretreatment biopsy must be done on the selected injection site (Lesion A) and the non-injection site (Lesion B),C1D1 and C3D1 prior to APX005M injection.

[l] Post injection Biopsy must be performed on Lesion A (the injection site) 24 hours (±7 hours) after the first APX005M injection.

[m] Mandatory tumor biopsies on Lesion A and, if possible, Lesion B. If Lesion A or Lesions A and B are able to be obtained, the tissue collection must be completed prior to Pembrolizumab administration cycle 5. Optional biopsy can be done at EOT, or if they progress with therapy.

[n] Pembrolizumab will be administered on Day 2 of Cycle 1, Cycle 2, Cycle 3, and Cycle 4. Pembrolizumab will be administered on Day 1 of Cycle 5 and thereafter.

[o] Only to be completed at Cycle 5 and Cycle 8.

[p] Starting Cycle 2 Day 1 See Section 4.2 for criteria details.

[q] Follow-Up assessments apply to Cycles 5-8 (Pembrolizumab at MDACC). Follow-Up assessments after Cycle 8 (receive Pembrolizumab outside MDACC) will occur every 3 months (every 12 weeks thereafter as applicable) and will include only Radiological Studies (at MDACC). See Section 3.4.4

[r] The APX005M initial injection can occur up to 2 days post the C1D1 physical exam, but prior to the first study dose of Pembrolizumab.

Appendix 3: Injection Site Patient Diary

Subject Name: _____

Lesion Location: _____

Date of Injection: _____

		Please provide a score of 0 to 10 for each of the following for each day following your injection 0 = None, and 10 = Very bad					
	Date	**Pain**	**Swelling**	**Redness**	**Itching**	**Warmth**	**Other**
Day 1	____/_____/____						
Day 2	____/_____/____						
Day 3	____/_____/____						
Day 4	____/_____/____						
Day 5	____/_____/____						
Day 6	____/_____/____						
Day 7	____/_____/____						