

Bispecific NK engager AFM13 Combined With NK Cells for Patients With Recurrent or Refractory CD30 Positive Hodgkin or Non-Hodgkin Lymphomas

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Protocol Body

1.0 Objectives

Primary objective:

Phase I part:

To establish the safety and recommended Phase II dose of umbilical cord blood (CB)-derived natural killer (NK) cells preloaded with the bispecific antibody AFM13 (AFM13-NK), followed by intravenous AFM13 in patients with refractory/relapsed CD30-positive lymphoid malignancies based on incidence of DLTs per dose level

Phase II part

To assess the activity of umbilical cord blood (CB)-derived natural killer (NK) cells preloaded with the bispecific antibody AFM13 (AFM13-NK), followed by intravenous AFM13 in patients with refractory/relapsed CD30-positive lymphoid malignancies. based on overall response rate (ORR), complete response (CR) rate and partial response (PR) rate.

Secondary Objectives:

1. To evaluate the duration of response
2. To evaluate the event-free survival (EFS) rate
3. To evaluate the overall survival (OS) time
4. To quantify the persistence of infused donor CB AFM13-NK cells in the recipient
5. To conduct comprehensive immune reconstitution studies

2.0 Background

2.1. CD30-positive lymphoid malignancies.

CD30 is a cell membrane protein of the tumor necrosis factor receptor family. It is universally expressed on classical Hodgkin lymphoma (HL) -which is a B-cell malignancy-, and systemic anaplastic large-cell lymphoma (ALCL) -a nodal T-cell lymphoma-. It is also frequently present in other nodal T-cell lymphomas, such as peripheral T-cell lymphoma not otherwise specified (PTCL NOS) and angioimmunoblastic T-cell lymphoma (AITL), as well as in cutaneous T-cell lymphomas (CTCL).

Results of current treatment of those malignancies vary widely. In cHL, the most common CD30-positive lymphoma, frontline chemotherapy ± radiotherapy is highly successful. However, up to 30% of patients are refractory to treatment or relapse.

Around 50-65% of these patients can be cured with salvage treatment followed by high-dose chemotherapy and autologous stem-cell transplant (ASCT).

With respect to nodal T-cell lymphomas (PTCL-NOS, ALCL and AITL), first-line treatment with CHOP or CHOP-like regimens results in poor outcomes, except for ALK+ ALCL. Despite intensified approaches in frontline therapy, such as consolidation with ASCT, these patients are still at considerable risk of relapse or early progression.

The rare and heterogeneous group of CTCL includes mycosis fungoides (MF) and Sezary syndrome (SS) as its most common forms. In early stages of CTCL, skin-directed therapy often effectively controls symptoms. However, for advanced MF and SS no curative therapies exist.

2.2. CD30-targeting therapies

CD30 is present, in varying degrees of prevalence, on the malignant cells of those tumors, and is, at the same time, generally not found on healthy tissues. Therefore, it constitutes an attractive therapeutic target. While naked anti-CD30 antibodies have shown minimal activity,[1] brentuximab vedotin (BV), an anti-CD30 antibody-drug conjugate with monomethyl auristatin E, a synthetic antineoplastic agent which inhibits cell division, presents remarkable activity in cHL [2] (for example, as part of frontline therapy [3], as maintenance after autologous stem-cell transplant [4], or for relapsed/refractory disease [2]), CD30+ ALCL and PTCL (as part of frontline therapy [5], or for relapsed/refractory tumors [6]), and CD30+ cutaneous T-cell lymphoma.[7]

In addition, CD30 is also an attractive target for novel cellular immunotherapeutic strategies that build on the remarkable success of immunotherapy in recent years in the aforementioned lymphomas, most notably, in cHL.

2.3. Expanded umbilical cord blood NK cells

NK cells are part of the innate immune system and have been implicated in tumor immunity.[8] They do not require prior exposure or sensitization to kill a specific target. While the exact mechanism of NK cells anti-tumor immunity remains unclear, a complex interplay between activating and inhibitory receptors determines cytotoxicity against a specific target.[9] This includes possible disinhibition of the killer immunoglobulin like receptor (KIR) due to absence of HLA class I molecules on target cells ("missing-self mechanism"), overexpression of stress ligands by tumor cells recognized by activating NK cell receptors (e.g., NKG2D, NKp30, NKp44, or NKp46), or binding of tumor antigen-specific antibodies to CD16a on the NK cells, which elicits antibody-dependent cellular cytotoxicity (ADCC) mediated by NK cells.[10]

It has been shown that NK cell populations are absent in the immunosuppressive TME of HL.[11] Furthermore, NK cells from patients with HL are dysfunctional due to, among other causes, an unfavorable balance between activating and inhibitory receptors.[12] Because of these limitations of autologous NK cell function, optimal NK immunotherapy for HL probably requires an allogeneic source.

The clinical safety of peripheral blood-derived allogeneic NK cell infusions has been

demonstrated.[13, 14] This requires collection of peripheral blood from a normal donor to generate NK cells, which can be logistically cumbersome. To minimize obstacles to collection, we have been interested in NK cells derived from cryopreserved umbilical cord blood (CB), a known source of hematopoietic progenitor cells and an “off-the-shelf” product. Our group has developed a Good Manufacturing Practices (GMP)-grade method of NK cell expansion from thawed CB mononuclear cells based on artificial antigen-presenting cells (Figure 1). This method yields a >1,000-fold expansion of NK cells with in vitro and in vivo antitumor activity.[15] Pre-activation of NK cells overnight with the combination of IL-18, IL-15 and IL-12 generates long-lived, memory-like NK cells, with greater effector function and ADCC.

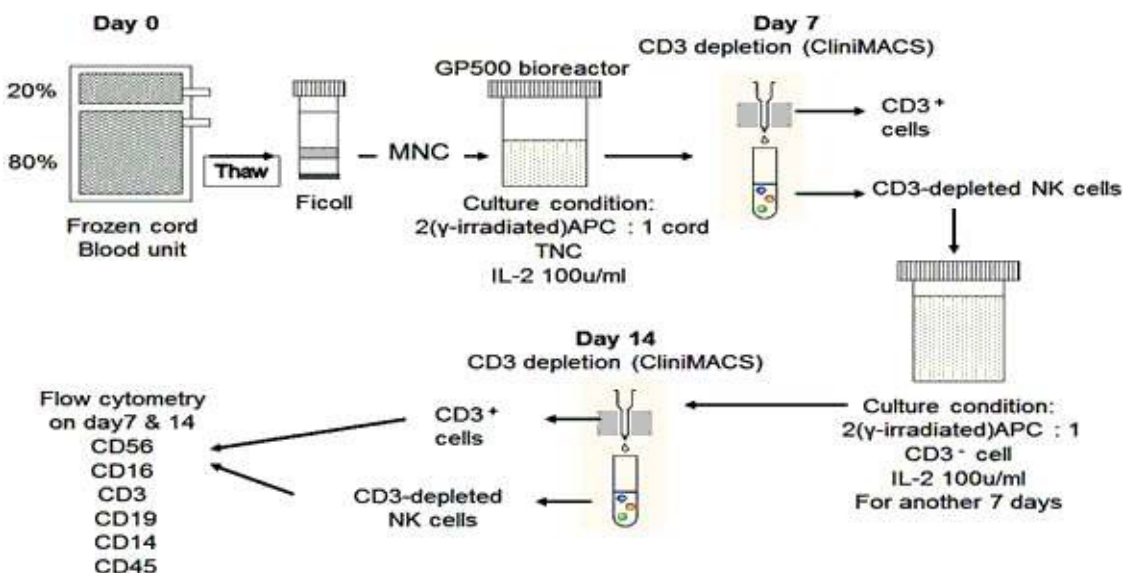


Figure 1. GMP-compliant method of expansion of CB NK cells.

We have pursued the application of allogeneic NK cells as adoptive cellular therapy for various hematological malignancies (MDACC protocols 2011-0379, 2014-0297, 2015-0571, and 2017-0295) and have demonstrated their safety with early observations of enhanced efficacy when compared to historical control patients who received similar treatments without NK cells. Doses of CB-NK cells of up to 10^8 cells/kg can be reliably produced for clinical use and are well tolerated.[16]

2.4. AFM13

AFM13 is a tetravalent chimeric antibody construct (TandAb) bispecific for CD30 and CD16A. It specifically targets CD30 on HL cells and other lymphomas and recruits and activates CD16A-positive innate immune cells such as NK cells and macrophages.[18] The addition of AFM13 to NK cells from HL patients results in a restoration of cytotoxicity of those functionally impaired NK cells.[17] AFM13 presents several important advantages over other CD30-targeting immune therapies, including other bispecific antibodies: 1) AFM13 does not bind to CD16B, which is expressed on granulocytes and may act as a sink for non-CD16A-specific antibodies, resulting in decreased efficacy; 2) AFM13 recognizes both allelic polymorphisms of CD16A, which guarantees an expanded patient population that might benefit from it (in contrast to, for instance, the case with rituximab or trastuzumab, both of which are significantly more active in patients homozygous for the high affinity allotypes of CD20 and HER2, respectively); and 3) Its

binding to CD30 and to CD16A, in a bivalent fashion with each of them, increases its affinity for them. Furthermore, it could be demonstrated that the unique epitope of the CD16A antibodies used in AFM13 is less prone to competition with physiological levels of circulating serum IgGs that compete with the Fc-binding of therapeutic monoclonal antibodies such as rituximab or trastuzumab. As a result, AFM13 results in increased cytotoxic potency and efficacy against CD30+ cells compared to native or Fc-enhanced anti-CD30 IgG or bivalent CD30/CD16A bispecific antibodies (Figures 2-A and 2-B).

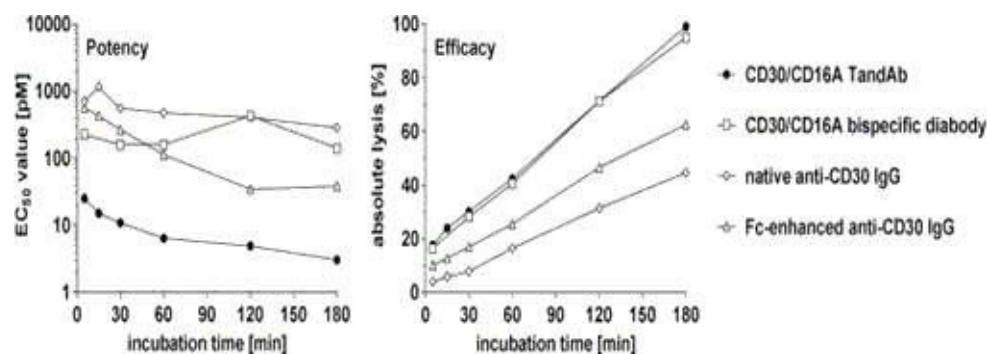


Fig. 2-A

Fig. 2-B

Figure 2. Comparison of AFM13 (CD30/CD16A TandAb) and CD30/CD16A bispecific antibodies, anti-CD30 IgG and Fc-enhanced anti-CD30 IgG in cytotoxicity assays. Fig. 2-A. Kinetics – The cytotoxic potency (EC₅₀) of the CD30/CD16A bispecific antibody and the Fc-enhanced IgG is 10–15-fold lower than that of the TandAb. Fig. 2-B. The cytotoxic efficacy (maximal cell lysis) of the Fc-enhanced IgG was more than 2-fold less than those of the similarly efficacious TandAb and diabody.

In addition, in the absence of CD30 binding, AFM13 does not activate NK cells, which minimizes the risk of off-target serious side effects from nonspecific NK activation and release of cytokines such as TNF- α or IFN- γ .

Rothe and collaborators reported a phase 1 trial of AFM13 in 28 patients with refractory or relapsed HL.[18] Patients received weekly doses of 0.01 to 7 mg/Kg for four weeks. Patients were heavily pretreated with a median of 6 (3-11) treatment lines, including prior ASCT in 22 patients (79%) and prior brentuximab vedotin in 9 patients (in 7 as most recent therapy, with PD in all of them). AFM13 was safe and well tolerated, and the maximally tolerated dose (MTD) was not reached. Grade 1-2 fever and chills were the most common side effects, which were easy to manage without need for premedication. Systemic exposure of AFM13 increased with escalating doses, and its plasma half-life was 19 hours. The ORR was 11.5% (all PRs), with an additional 50% SD rate for an overall disease control rate of 61.5%. Tumor shrinkage was seen in 3 of 7 patients refractory to their most recent treatment with brentuximab vedotin, indicative of non-cross-resistance between both CD30-targeting therapies. Immediately after infusion of AFM13 there was a transient decrease of circulating NK cells and an increase of circulating activated NK cells (CD69+), consistent with tissue recruitment and activation of NK cells. The concentration of soluble CD30 (sCD30) decreased 24 hours after the infusion.

Sawas and coworkers reported at the 2018 ASH Meeting a phase 1 trial of AFM13 in

patients with relapsed or refractory CD30-positive lymphoma with cutaneous presentation.[19] Nine patients received 8 doses of AFM13 administered in 3 different schedules (1.5 mg/Kg IV weekly, 7.5 mg/Kg IV weekly, and 7 mg/Kg continuous infusion IV over 5 days weekly). The median number of prior therapies was 4 (range, 1-11), 2 patients had progressed on brentuximab vedotin and 5 patients had failed total skin electron beam radiotherapy. The ORR was 50% with 1 CR and 3 PRs. The authors observed a decrease in circulating NK cells during treatment with recovery 3 weeks after end of treatment. Patients who responded had an increase of the activation marker CD69 on NK cells, increased infiltration of tumor biopsies with CD56+ NK cells, and a decrease of circulating Tregs, as compared with non-responders.

At the same 2018 ASH Meeting Bartlett et al. presented a phase 1 trial of the combination of escalating doses of AFM13 with pembrolizumab (200 mg IV every 3 weeks) in patients with relapsed or refractory HL after failing brentuximab vedotin.[20] Thirty patients, pretreated with a median 4 (3-7) therapy lines including brentuximab vedotin (which was the latest therapy for 43% of them), 37% with prior ASCT, were enrolled. The dose-limiting toxicity was 1 case of grade 4 infusion-related reaction (IRR). The most common adverse events were IRR (80%), rash (30%), pyrexia (23%), nausea (23%), diarrhea (20%), fatigue (17%), headache (17%), and elevation of transaminases (10%). Of them, grade 3-4 toxicities included IRR (13%), elevation of transaminases (3%), gastritis (3%), hypotension (3%), nausea/vomiting (3%), and neutropenia (3%). None of these adverse events led to treatment discontinuations. The ORR and CR rates were very encouraging at 87% and 35%, respectively.

2.5. In vitro studies of the combination of AFM13 with CB-derived NKcells

Mononuclear cells were isolated from fresh or frozen CB units by ficoll density gradient centrifugation. CD56+ NK cells were cultured with rhIL-12, rhIL-18 and rhIL-15 for 16 hours, followed by ex vivo expansion with rhIL-2 and irradiated (100 Gy) K562-based feeder cells expressing membrane-bound IL-21 and CD137-ligand (2:1 feeder cell:NK ratio). After 14 days, NK cells were loaded with serial dilutions of AFM13 (0.1, 1, 10 and 100 mg/ml). After washing twice with PBS, we tested the effector function of AFM13-loaded NK-cells (AFM13-NK) compared to expanded CB-NK cells without AFM13, against Karpas-299 (CD30 positive) and Daudi (CD30 negative) lymphoma cell lines by ⁵¹Cr release and intracellular cytokine production assays. Karpas-299 and Daudi cell lines were labeled with ⁵¹Cr for 2 hours at 37°C, washed, and resuspended. Effector cells were used at an effector-to-target (E:T) ratios of 40:1 to 1.25:1 in presence of 100 µg/ml AFM13 or tested in 5:1 ratio using different concentration of AFM13 for 4 hours. Spontaneous lysis was determined by measuring ⁵¹Cr-release into the supernatant on a gamma-counter. Spontaneous release was assessed by incubating target cells alone, maximum lysis by addition of 1% Triton X-100 (Sigma-Aldrich). Specific ⁵¹Cr / lysis was determined following the equation: Percentage of specific lysis (%) = 100 x (Test release - Spontaneous release) / (Maximal release - Spontaneous release).

AFM13-NK cells killed Karpas-299 cells more effectively at all E:T ratios tested than unloaded NK cells (Figure 3) and produced statistically more IFN-α and CD107a (P=0.0034; P=0.0031 respectively, n=4). In contrast, AFM13-NK cells and unloaded NK cells exerted similar cytotoxicity against Daudi cells.

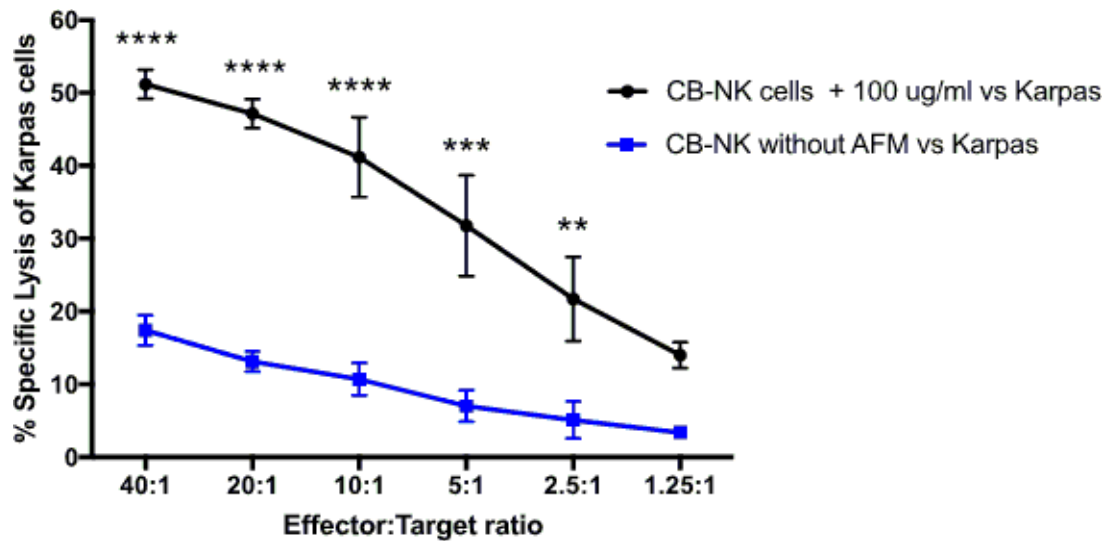


Figure 3. Karpas-299 cells incubated at a fixed ratio with expanded CB-NK or with preactivated and expanded (P+E) CB-NK cells loaded with 100 $\mu\text{g}/\text{mL}$ of AFM13. Cytotoxicity was determined after 4 hours using a ^{51}Cr release assay.

We next tested the stability of AFM13 binding to the NK cells by washing the NK cells twice with PBS following a 1-hour incubation with AFM13. In this experiment, human NK cells were incubated with increasing concentrations of AFM13 (0.001-100 $\mu\text{g}/\text{mL}$) for 1 hour, with or without a post-wash step and their cytotoxicity against Karpas 299 was determined directly by ^{51}Cr -release assay. As shown in Figure 4, regardless of the AFM13 concentration used, incubation with AFM13 for 1 hour ensured efficient loading of NK cells with AFM13; AFM13-loaded and washed were just as efficient at killing Karpas 299 targets as the loaded and unwashed NK cells (Figure 4).

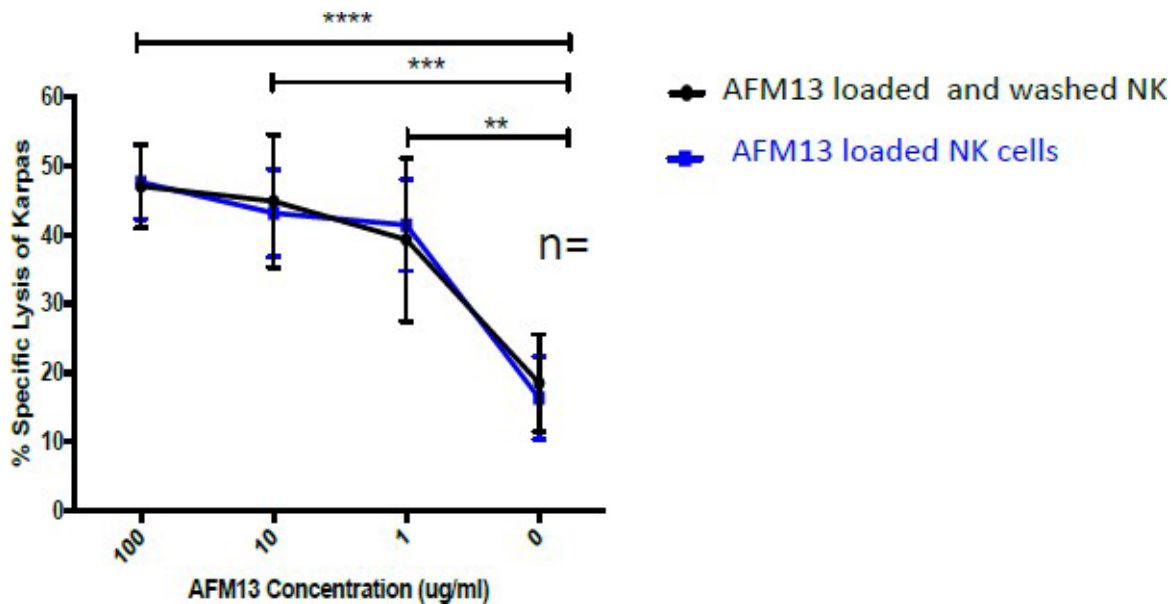


Figure 4. Preactivated and expanded (P+E) CB-NK cells were incubated with increasing concentrations (1, 10 and 100 µg/ml AFM13) for 1 hour. NK cells were then washed twice with PBS and their cytotoxicity measured against Karpas-299 cells, in a 4-h ^{51}Cr assay at an E:T ratio of 10:1

Next, we established 100 µg/ml as the optimal concentration of AFM13 for loading (Figure 4) and 1 hour as the optimal incubation time to obtain maximal activity in a series of *in vitro* experiments.

To gain insight into the phenotypic changes of AFM13 loaded vs. unloaded NK cells, we performed flow cytometric analysis of NK surface receptors. Expression of activating receptors, such as CD69, CD25 or TRAIL, was induced on the AFM13-loaded (but not on the unloaded) NK cells, both in the presence and absence of Karpas-299 cells. Importantly, AFM13 expression was detectable on the surface of NK cells for up to 7 days (Figure 5).

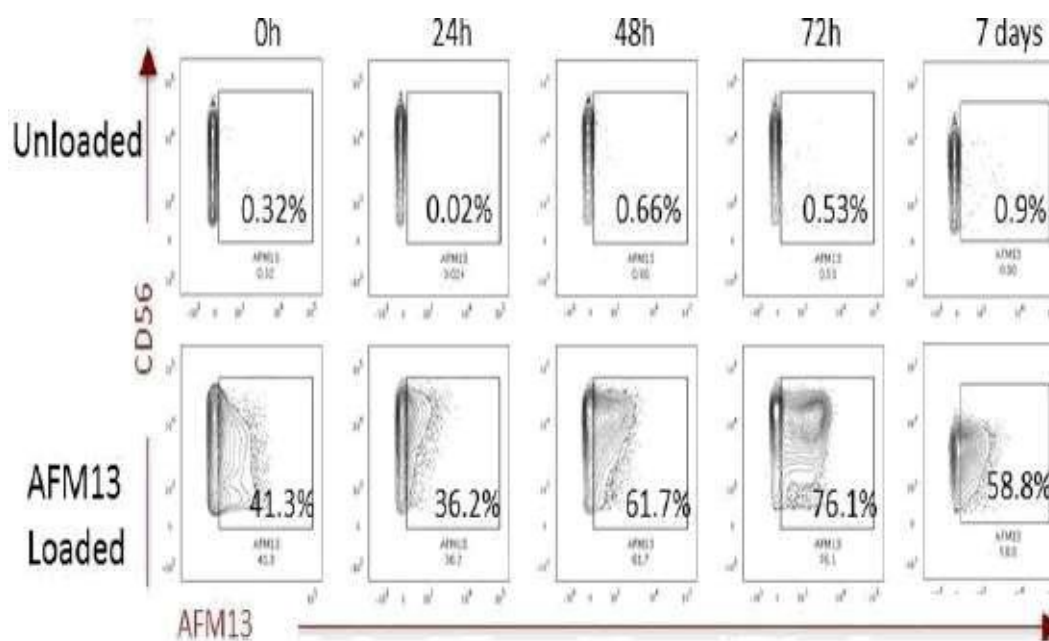


Figure 5. Cell surface retention of AFM13 on the surface of preloaded CB NK cells for up to 7 days

Retention of AFM13 on NK cell surface enhanced cytotoxicity against CD30-positive Karpas-299 (Fig. 6-A), but not CD30-negative Daudi cells (Fig. 6-B).

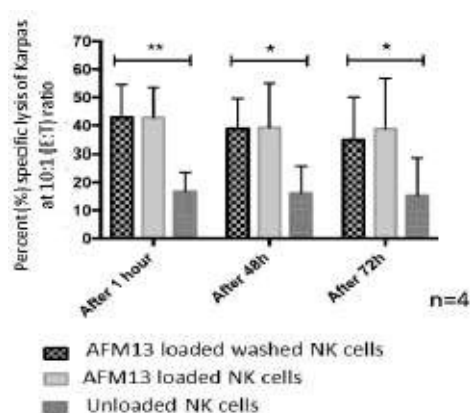


Fig. 6-A

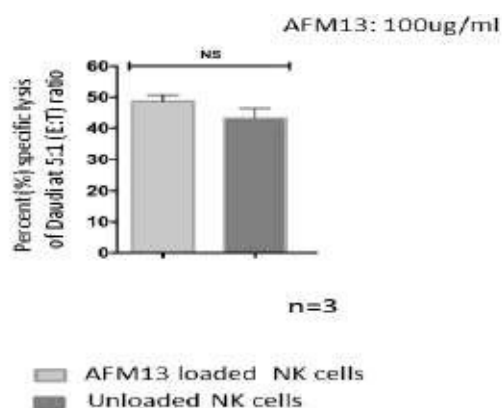


Fig. 6-B

Figure 6. Effect of AFM13-loaded and unloaded NK cells on CD30-positive Karpas-299 cells (Fig. 6-A) and CD30-negative Daudi cells (Fig. 6-B).

2.6. In vivo murine studies of AFM-loaded CB-NK cells.

Karpas-299 is a lymphoma cell line resistant to NK killing, but when the NK cells are loaded with AFM13, the NK cells can then kill them. Thus, NSG null mice, injected intravenously with Karpas-299 cells, is a valid murine model to study the cytotoxicity of AFM13-loaded NK cells in vivo (Figure 7).

Group Categories: 5 mice/group, 4 groups and 20 mice total:

- Group #1: Karpas-299-ffluc.
- Group #2: Karpas-299-ffluc + NK cells.
- Group #3: Karpas-299-ffluc + NK cells loaded with AFM13.
- Group #4: Karpas-299-ffluc + Injection of AFM13.

Doses:

- Tumor cells: Group #1-4 Karpas 299-ffluc. Dose of Karpas: 0.1×10^6 /mouse.
- Effector cells: Groups #2-3: P+E cells - 10×10^6 cells/mouse.
- AFM13: Groups #3-4: 100 μ g AFM13, as determined in our in vitro experiments.

Route of administration: Intravenously through the tail vein.

Timing of postmortem assessments: Weekly from day 7 with bioluminescence imaging and flow cytometry.

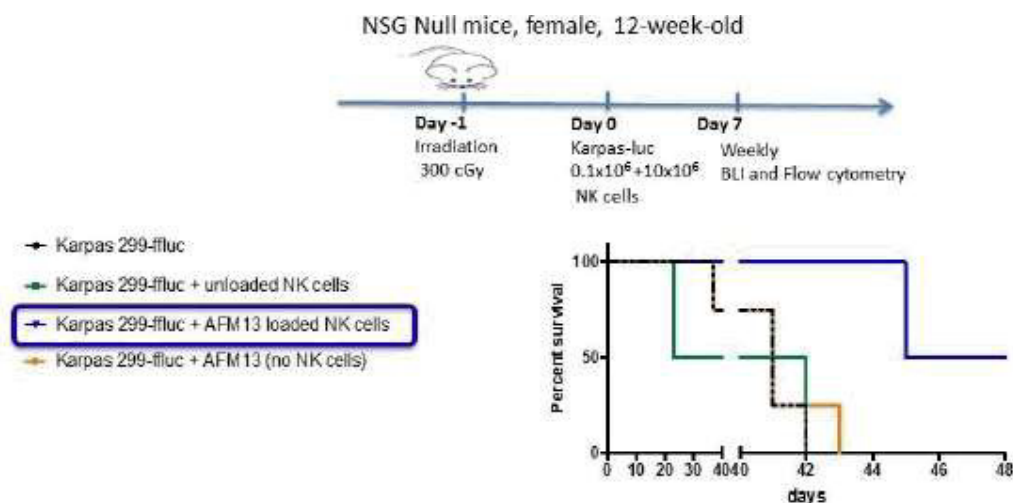


Fig. 7-A

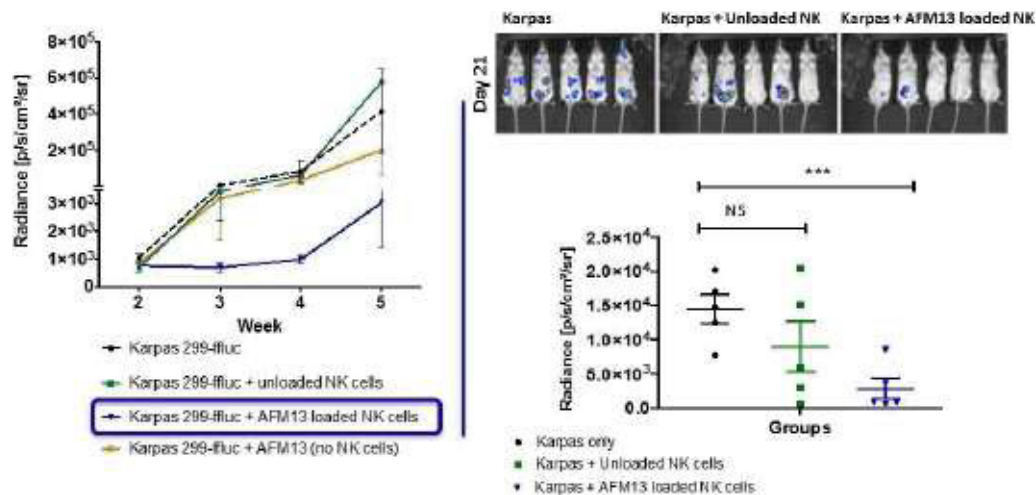


Fig. 7-B

Figure 7. NSG null mice were injected intravenously with luciferase-transduced Karpas-299 cells (Karpas299-ffluc) and treated intravenously with AFM13-loaded NK cells, unloaded NK cells and AFM alone (Figs. 7-A and 7-B).

Flow cytometry studies of sacrificed mice showed AFM13-loaded NK cells homed to the sites of tumor (liver, spleen and bone marrow) but were not detectable in peripheral blood. This was determined by flow cytometry (CD56+CD3- population) in the harvested organs.

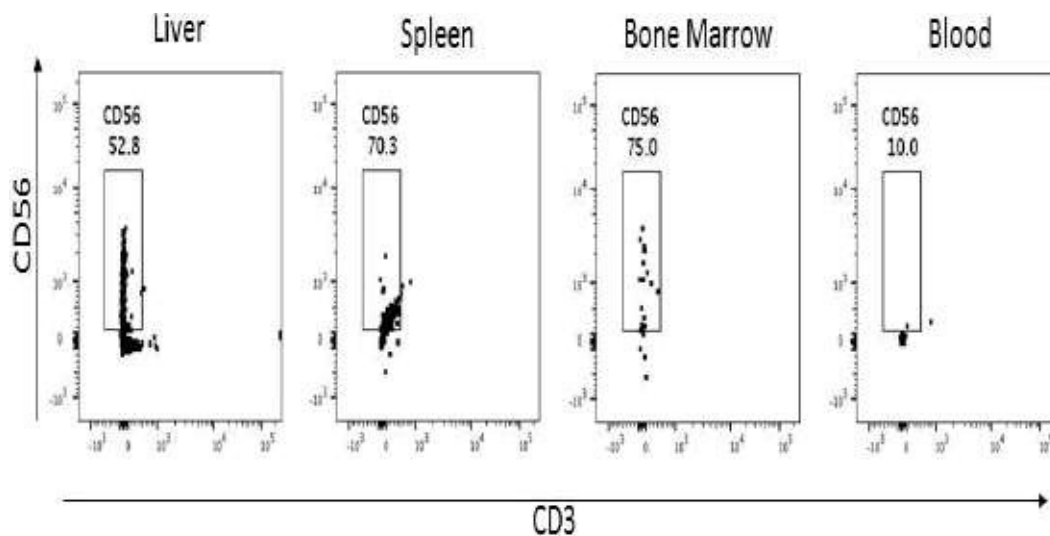


Figure 8. Homing of AFM13-loaded CB-NK cells to the liver, spleen and bone marrow, but not blood (day 18 of treatment).

Building on these preclinical experiments and our prior research with expanded CB-derived NK cells, we aim to develop a novel cellular product for adoptive therapy with AFM13-loaded CB-NK cells. We intend to conduct a trial of expanded “off the shelf” umbilical CB-NK cells combined with AFM13 in patients with refractory HL and other CD30-positive lymphoid tumors. We hypothesize that this novel adoptive immunotherapeutic approach will be safe and effective against these malignancies.

3.0 Patient Eligibility

Inclusion Criteria:

1. Patients with a diagnosis of relapsed or refractory classical HL, anaplastic large cell lymphoma (ALCL), peripheral T-cell lymphoma not otherwise specified (PTCL-NOS), mycosis fungoides (MF), or B-cell non-Hodgkin lymphoma with a pre-enrollment tumor biopsy positive for CD30 by immunohistochemistry at $\geq 1\%$. Patients with HL, ALCL and MF must be refractory or intolerant to brentuximab vedotin.
2. Age 18 - 75 years (15 - 75 years once the MTD has been determined).
3. Karnofsky performance status $\geq 60\%$
4. Absolute neutrophil count $\geq 500/\text{mm}^3$ and platelet count $\geq 50,000/\text{mm}^3$
5. Adequate renal function, as defined by estimated serum creatinine clearance ≥ 50 ml/min, estimated using the Cockcroft-Gault equation: Estimated creatinine clearance = $(140 - \text{age [years]}) \times \text{weight (kg)} \times \text{Fa} / \text{serum creatinine (mg/dL)} \times 72$ [a where $F=0.85$ for females and $F=1$ for males.]
6. Adequate hepatic function (ALT and/or AST $\leq 3 \times \text{ULN}$; bilirubin and ALP $\leq 2 \times \text{ULN}$. Patients with cancer involvement of the liver and elevation of ALT, AST, bilirubin, and/or ALP $\leq 5 \times \text{ULN}$ are eligible, per PI discretion.
7. Adequate pulmonary function with FEV1, FVC and DLCO (corrected for Hgb and volume) $\geq 50\%$.
8. Adequate cardiac function with left ventricular ejection fraction $\geq 40\%$. No uncontrolled arrhythmias or symptomatic cardiac disease.
9. If female of child-bearing potential, must not be pregnant or be breastfeeding and required to have a negative urine or serum pregnancy test prior to enrollment.

[Note: Urine pregnancy tests that cannot be confirmed as negative, require a confirmatory negative serum pregnancy test]. In addition, females of childbearing potential must agree use of a highly effective method of contraception (Section 10.0.) for the course of the study from 14 days prior to the first dose of study drug until 60 days after the last dose of study drug. Non-childbearing potential is defined as:

- Postmenopausal: defined as no menses for 12 months without an alternative medical cause. A high follicle-stimulating hormone (FSH) level in the postmenopausal range may be used to confirm post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, FSH measurements

indicating post-menopausal status must be documented in patient's medical history.

- Permanently sterile: documented permanent sterilization e.g. hysterectomy, bilateral salpingectomy and bilateral oophorectomy. If male, surgically sterile or agrees to use a highly effective method of contraception (Appendix 1), 14 days prior to the first dose of study drug until 60 days after the last dose of study drug].

Exclusion criteria:

1. Major surgery <4 weeks prior to first dose of study drug
2. Any other severe or uncontrolled disease or condition which might increase the risk associated with study participation
3. Any other malignancy known to be active, with the exception of treated cervical intra-epithelial neoplasia and non-melanoma skin cancer.
4. Grade ≥ 3 non-hematologic toxicity from prior therapy that has not resolved to grade ≤ 2
5. Active hepatitis B, either active carrier (HBsAg +) or viremic (HBV DNA $\geq 10,000$ copies/mL, or $\geq 2,000$ IU/mL), or hepatitis C (detectable viral load by HCV RNA PCR)
6. Active infection requiring parenteral antibiotics
7. HIV infection
8. Treatment within prior 2 weeks with any anti-cancer agent, investigational or approved
9. Active central nervous system (CNS) involvement (untreated parenchymal brain metastasis or positive cytology of cerebrospinal fluid)
10. Life expectancy ≤ 6 months
11. Previous treatment with AFM13

4.0 Treatment Plan

On day -15 CB NK cell preactivation and expansion will begin as described in detail in the MD Anderson GMP Laboratory standard operating procedure (SOP). If needed, NK cells that have been cultured up to 22 days can be infused. Patients will be admitted on day -6 to start standard lymphodepleting chemotherapy the next day: fludarabine 30 mg/m² and cyclophosphamide 300 mg/m² on days -5 to -3 (Table 1). Systemic corticosteroid therapy must be discontinued ≥ 7 days prior to the first dose of study drug.

On day 0 the preactivated and expanded CB NK cells will be washed and combined with AFM13 at 100 μ g/mL, as described in the GMP Laboratory SOP; the AFM13-NK cell product will then be washed and infused. The dose of NK cells of cycle 1 are shown in Table 2. NK Cell-AFM13 will be infused via dedicated line, free flowing and with no filter, for a duration of 30 minutes

On days +7, +14 and +21 the patient will receive an intravenous infusion of AFM13 alone at 200 mg over 4 hours

D-5 through D-2 may be given on an outpatient basis, with admit/IV hydration on D-1, at the discretion of the attending physician.

Patients will be monitored approximately every 15 minutes for 1 hour then if stable every 30 minutes thereafter until end of each AFM13 and AFM13-NK infusion.

Patient may be discharged after the end of the D0 infusion at the discretion of the attending physician.

Treatment Plan 1.a. - Inpatient

On or Before D-15	Begin CB NK cell production incubated with AFM13
D-6	Admit / IV hydration
D-5	Fludarabine 30 mg/m2 IV / Cyclophosphamide 300 mg/m2 IV
D-4	Fludarabine 30 mg/m2 IV / Cyclophosphamide 300 mg/m2 IV
D-3	Fludarabine 30 mg/m2 IV / Cyclophosphamide 300 mg/m2 IV
D-2	Rest
D-1	Rest
D 0	AFM13 + CB NK Infusion
D+7	AFM13 200 mg
D+14	AFM13 200 mg
D+21	AFM13 200 mg

Treatment Plan 1.b. - Outpatient

On or before D-15	Begin CB NK cell production incubated with AFM13
D-5	Fludarabine 30 mg/m2 IV / Cyclophosphamide 300 mg/m2 IV
D-4	Fludarabine 30 mg/m2 IV / Cyclophosphamide 300 mg/m2 IV
D-3	Fludarabine 30 mg/m2 IV / Cyclophosphamide 300 mg/m2 IV
D-2	Rest
D-1	Admit / IV hydration
D 0	AFM13 + CB NK Infusion
D+7	AFM13 200 mg
D+14	AFM13 200 mg
D+21	AFM13 200 mg

Fludarabine and cyclophosphamide doses will be based on actual body weight.

No dose adjustments for renal and/or hepatic impairment related to fludarabine will be made in this study.

G-CSF (filgrastim-sndz, Zarxio) may be administered per institutional guidelines

if clinically indicated.

Treatment Plan 2.a and 2.b will be used if fludarabine is not available due to shortages and for patients with prior neurotoxicity or any other contraindication to the use of fludarabine.

Treatment Plan 2.a. - Inpatient

On or Before D-15	Begin CB NK cell production incubated with AFM13
D-6	Admit / IV hydration
D-5	Cladribine 5 mg/m2 IV / Cyclophosphamide 300 mg/m2 IV
D-4	Cladribine 5 mg/m2 IV / Cyclophosphamide 300 mg/m2 IV
D-3	Cladribine 5 mg/m2 IV / Cyclophosphamide 300 mg/m2 IV
D-2	Rest
D-1	Rest
D 0	AFM13 + CB NK Infusion
D+7	AFM13 200 mg
D+14	AFM13 200 mg
D+21	AFM13 200 mg

Treatment Plan 2.b. - Outpatient

On or before D-15	Begin CB NK cell production incubated with AFM13
D-5	Cladribine 5 mg/m2 IV / Cyclophosphamide 300 mg/m2 IV
D-4	Cladribine 5 mg/m2 IV / Cyclophosphamide 300 mg/m2 IV
D-3	Cladribine 5 mg/m2 IV / Cyclophosphamide 300 mg/m2 IV
D-2	Rest
D-1	Admit / IV hydration
D 0	AFM13 + CB NK Infusion
D+7	AFM13 200 mg
D+14	AFM13 200 mg
D+21	AFM13 200 mg

Around day +28 the patient will undergo restaging of the tumor, including a repeat PET/CT scan and a bone marrow exam (if previously involved).

Patients who do not show progression after their first cycle will receive a second cycle at the same dose level. For patients who show an objective response after the second cycle, additional cycles can be given as clinically indicated (e.g. to consolidate a CR or to convert a PR into a CR) if feasible, based on the patient's general condition and availability of NK cell product

Table 2. Dose escalation of CB NK cells.

Cohort	AFM13-NK Cell Dose	Number of patients
1	Up to 1×10^6 per Kg	3-6
2	Up to 1×10^7 per Kg	3-6
3	Up to 1×10^8 per Kg	3-6

5.0 Study Design

This is a combined phase 1/ phase 2 study.

In the first part of the study the recommended dose of NK cells in combination with AFM13 will be established. If no DLTs occur up to the highest cell dose of 1×10^8 after 6 patients, this dose will be defined as recommended phase 2 dose and the efficacy of this dose in patients with HL and CD30+ NHL will be assessed.

The aim is to recruit a total of 30 response evaluable patients with HL and 10 response evaluable patients with CD30+ NHL at this dose level. Patients from the dose escalation part of the study who are response evaluable and treated at the recommended phase 2 dose, will be counted towards the final number of patients

Table 2. Dose escalation of CB NK cells.

Cohort	AFM13-NK Cell Dose	Number of patients
1	Up to 1×10^6 per Kg	3-6
2	Up to 1×10^7 per Kg	3-6
3	Up to 1×10^8 per Kg	3-6

We will enroll and treat patients in cohorts of size 3. DLT will be evaluated for each patient on day 28. The last two subjects in the 1st cohort and all three subjects in the 2nd cohort will wait for all prior patients to reach day 28 DLT assessment before being enrolled.

August 2021 update: 3 patients have been treated in each of the first two dose levels with no DLT. A total of 3 patients have been treated at the third dose level. None of these patients experienced DLT by day 28, we will continue to follow the algorithm detailed in Section 10, but we will not wait for a cohort of patients to be fully evaluated before continuing with additional cohorts. If more than 1 patient experiences DLT, we will halt enrollment pending full evaluation of all patients at day 28 before proceeding.

We will exclude minors (ages < 18) from the phase 1 dose escalation portion of the protocol. Once the MTD has been determined after the first 9 adult patients, minors (ages 15 to 18) will be eligible in the phase 2.

6.0 Evaluation During Study

The schedule of evaluations is shown on **Table 3**. Every effort will be made to adhere to the schedule of events and all protocol requirements. Variations in schedule of events and other protocol requirements that do not affect the rights and safety of the patient will not be considered as deviations. This decision will be made by the PI. Such variations may include laboratory assessments completed outside of schedule and occasional missed required research samples. Missed samples for correlative studies will not constitute protocol deviations.

Patients will be followed up on study for 1 year or until progression or subsequent therapy whichever is shorter. If the patient subsequently undergoes SCT transplant, we will continue to follow the patient on study for 1 year post transplant.

Table 3. Evaluation schedule

2018-1092	Pre-treatment (within 30 days of enrollment)	Day 0	Day 1	Day 2 (+/- 1 day)	Day 7 (+/- 2 days)	Day 9 (+/- 2 days)	Day 14 (+/- 2 days)	Day 21 (+/- 2 days)	Day 22 (+/- 2 days)	Week 4 (+/- 5 days)	Week 8 (+/- 5 days)	Day 100 (+/- 5 days)	Day 180 (+/- 14 days)	9 months (+/- 21 days)	1 year (+/- 21 days)
History and physical exam, weight and vital signs	X				X							X(1)	X(1)	X(1)	X(1)
Karnofsky Performance Status	X											X(1)	X(1)	X(1)	X(1)
Urine pregnancy test (within 3 days prior to 1st dose of study drug)	X														
CBC, total bilirubin, ALT, AST, alkaline phosphatase, LDH, albumin, total protein, BUN, creatinine, glucose, electrolytes, PT/PTT, immunoglobulin levels and cytokine analyses	X	X		X	X		X	X		X	X	X(1)	X(1)	X(1)	X(1)
Type and screen	X	X		X(1)	X		X	X		X	X	X(1)	X(1)	X(1)	X(1)
Serology for HIV, HBV, HCV	X														
ECHO or MUGA	X														
Pulmonary function tests	X														
Chest x-ray	X													X(1)	X(1)
Urinalysis	X														
Unilateral bone marrow aspiration (BMA) and biopsy	X				X (1)					X(1)		X (1)	X(1)	X (1)	X(1)
PET/CT scan	X									X		X(1)	X(1)	X(1)	X(1)
CT brain	X (1)														
Research Studies, blood (2)	X	X			X		X	X		X		X(1)			

2018-1092	Pre-treatment (within 30 days of enrollment)	Day 0	Day 1	Day 2 (+/- 1 day)	Day 7 (+/- 2 days)	Day 9 (+/- 2 days)	Day 14 (+/- 2 days)	Day 21 (+/- 2 days)	Day 22 (+/- 2 days)	Week 4 (+/- 5 days)	Week 8 (+/- 5 days)	Day 100 (+/- 5 days)	Day 180 (+/- 14 days)	9 months (+/- 21 days)	1 year (+/- 21 days)
PK Studies(3)		X	X	X	X	X	X	X	X						
Antidrug antibodies (ADA) (3)		X					X	X							
Serum CD30(3)		X	X					X	X						

- (1) If clinically indicated.
- (2) Research blood sample of 60 mL will be drawn after the consent is signed but before the lymphodepleting chemotherapy is initiated for Dr. Rezvani's lab. These studies are free of charge to the patient. See Research Samples subsection below regarding time point details.
- (3) See [Appendix 3](#), for details regarding timing and number of samples per time point. Repeat all samples if the patient receives Cycle 2; collected on Day 0 for Cycle 3 and beyond.

Baseline evaluations prior to start AFM13-NK production.

- To be performed within 30 days of study enrollment:
 - History and physical examination.
 - CBC, total bilirubin, SGPT, SGOT, alkaline phosphatase, LDH, albumin, total protein, BUN, creatinine, glucose, electrolytes, PT/PTT, type and screen, and immunoglobulin levels.
 - Serology for HIV, HBV and HCV.
 - ECHO or MUGA.
 - Pulmonary function tests.
 - Chest x-ray.
 - Urinalysis.
 - Urine pregnancy test.
 - Unilateral bone marrow aspiration (BMA) and biopsy.
 - PET/CT scan.
 - CT brain – if clinically indicated.

Research Samples

- Research blood sample of 60 mL will be drawn after the study consent is signed but before the start of lymphodepleting chemotherapy. These studies will be done at Dr. Rezvani's lab free of charge to the patient:
 - Research blood samples will be obtained at Baseline (prior to chemotherapy in Cycle 1 and Cycle 2, if applicable). Research blood samples will be obtained on day 0, day 2 (+/- 1 day), day 7 (30 minutes after the end of AFM13 infusion), day 8, day 14 (30 minutes after the end of AFM13 infusion), day 15, day 21 (30 minutes after the end of AFM13 infusion), day 22, week 4 (+/-5 days), and day 100 (+/- 5 days).

All research blood samples will be repeated if patient receives an additional treatment cycle.

Evaluation during Study (After AFM13-NK cell Infusion):

- The following evaluations will be obtained on day 0, day 2 (+/- 1 day), 7 (+/- 2 days), 14 (+/- 2 days), and 21 (+/- 3 days), week 4 (+/-5 days), 8 (+/-5 days),, day 100 (+/- 5 days), Day 180 (+/- 14 days), and at 9 months (+/- 21 days) and 1 year (+/- 21 days):
 - CBC, total bilirubin, SGPT, SGOT, alkaline phosphatase, LDH, albumin, total protein, BUN, creatinine, glucose, electrolytes, chimerism, cytokine analysis, type and screen (if clinically indicated)
 - Restaging: PET/CT, and unilateral BMA (if clinically indicated) on day +28 (+/- 3 days) of each cycle
- The following evaluations will be obtained on day 7 (+/- 2 days), 100 (+/- 5 days), 180 (+/-14 days), 9 months (+/- 21 days), and 1 year (+/-21 days):
 - Physical examination including weight and vital signs.
- The following evaluations will be obtained on week 4 (+/- 5 days), day 100 (+/-5 days), day 180 (+/- 14 days), and at 9 months (+/- 21 days) and 1 year (+/- 21 days):
 - PET/CT scans.
- The following evaluation will be obtained at 9 months (+/- 21 days) and 1 year (+/- 21 days) if clinically indicated:
 - Chest x-ray.

Additional lab tests:

- Additional lab draws may be needed to monitor the NK cells and patient disease status. The patient may be asked to sign a separate consent for protocol LAB00-099 for these additional samples. Analysis will be performed in Dr. Rezvani's lab free of charge for the patient.
- New lesions suspicious for relapse or progression will be considered for biopsy as clinically indicated. A sample of fresh tissue will be processed through the Institutional Tissue Bank for research studies.

Evaluation of responses will follow the Lymphoma Response to Immunomodulatory Therapy Criteria (LYRIC) for definition of CR, PR, progressive disease (PD) and indeterminate responses (IR).[21]

7.0 Background Drug Information

7.1. AFM13:

Pharmaceutical and therapeutic background

The investigational medicinal product AFM13 is a tetravalent bispecific chimeric (anti-human CD30 x anti-human CD16A) recombinant antibody construct that is being developed for the indication of HL and other CD30-positive malignancies.

AFM13 is constructed by means of recombinant technology as a homodimer of a polypeptide comprising four antibody variable domains, each one being separated from the neighboring domains by a glycine-serine linker of nine amino acids. The four-domain gene product is unable by itself to bind antigen, since the lengths of the linkers between the domains are too short for intramolecular pairing of the corresponding heavy and light chains. However, they are arranged in an order that permits formation of the corresponding antibody VH/VL domains after non-covalent dimerization. The result of this folding process is a homodimer molecule with a molecular weight of 104 Da (figure 9)

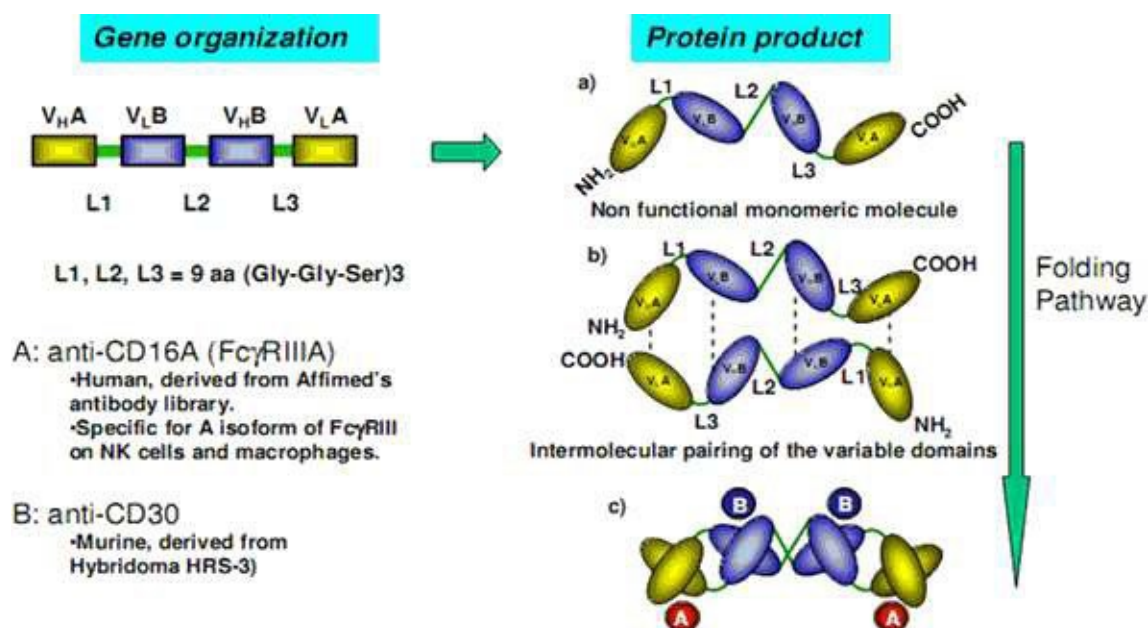


Figure 9: AFM13 structure

The tetravalent bispecific format, as represented by AFM13, is designed to recruit cytotoxic effector cells of the immune system effectively against tumor target cells.

NK-cells are such cytotoxic effector cells and represent a potent subset of lymphocytes for targeting and lysing tumor cells. In contrast to T lymphocytes, they do not need to be pre-activated. Their inherent cytolytic activity can be stimulated via the FcγRIIIA receptor (CD16A), which is expressed on the surface of NK cells, macrophages, and activated monocytes.

Nonclinical studies

The PK behavior of AFM13 has been investigated in stand-alone studies in mice, and in cynomolgus monkeys as the only relevant species. Furthermore, toxicokinetic data were generated in cynomolgus monkeys in parallel with the toxicological assessment of AFM13.

Characterization of affinity and specificity

The affinity and specificity of AFM13 to its nominal ligands CD30 and CD16A has been extensively characterized. The results demonstrate high affinity of AFM13 to both of its

ligands. AFM13 binds to human CD16A with an affinity of ~1.6 nM and to human CD30 with an affinity of ~1.3 nM. The experiments using different isoforms of CD16 also confirmed the specificity of AFM13 for CD16A and revealed comparable binding to both tested CD16A allelic variants. No binding of AFM13 to different variants of CD16B was detectable. Using flow cytometry, cell surface binding of AFM13 on CD30-positive and CD16-positive cells could be unequivocally demonstrated. The flow cytometry data using cell lines transfected with different CD16 isoforms also clearly confirmed specificity of AFM13 for the A isoform of CD16. CD30- and CD16-specific tissue binding of AFM13 could be well demonstrated. Staining patterns of AFM13 on selected frozen human tissue sections corresponded to those seen with monoclonal control antibodies directed against either CD16 or CD30.

Pharmacodynamic studies

In vitro AFM13 induces specific and selective killing of CD30-positive target cells by NK cells with an EC₅₀ typically seen in the order of ~5 – 250 pM (depending on the target cell line and effector cells used). AFM13-induced target cell lysis is specific since AFM13-activated killing of CD30-positive target cells by NK cells does not affect CD30-negative bystander cells. The onset of AFM13-mediated target cell lysis is immediate and does not require pre-activation of NK-cells. AFM13 which is already bound to CD30-positive target cells or CD16A-positive effector cells, is able to mediate target cell lysis even in the absence of free soluble AFM13.

Secondary pharmacodynamic properties of AFM13 were investigated in several *in vitro* systems. These *in vitro* studies encompassed the ability to induce cytokine release in human peripheral blood mononuclear cells (PBMCs) and the ability to induce proliferation in CD30-positive and CD16A-positive cells. The ability of free soluble AFM13 to induce cytokine release from human PBMCs is minimal. Nevertheless, it is not possible to claim that AFM13 is free of cytokine releasing potential. Measurement of plasma concentrations of IL-2, IL-6, TNF- α , and IFN- α , which are known to be most relevant for cytokine-related adverse reactions, did not indicate cytokine release in the pivotal 28 days repeated dose toxicity study in cynomolgus monkeys up to a dose level of 10 mg/kg.

Additional data do not indicate strong induction of cytokine release from human PBMC by immobilized AFM13 or AFM13 in the presence of physiological and non- physiological cross-linkers. The most effective cross-linker with regard to induction of cytokines (especially TNF- α and IFN- α) by AFM13 seem to be CD30-positive target cells. This may be part of the mode of action since activation of NK-cells is normally associated with release of IFN- α . Binding of AFM13 to CD16A-positive cells in cultures of human PBMC or binding to CD30-positive on HL cells does not trigger or affect proliferation.

Safety pharmacology

A number of safety pharmacology end-points concerning the CNS, cardiovascular system, respiratory system, gastrointestinal system, renal/urinary system, and hemostatic system, have been integrated into standard toxicity studies of AFM13 in cynomolgus monkeys. No warning signs with regard to safety pharmacology arose from these studies.

Tissue cross reactivity

In human non-epithelial tissues, the staining pattern shown by AFM13 is consistent with its specificity, detecting only CD16-positive and CD30-positive cells. However, AFM13 shows strong immunostaining of a number of secretory-type human epithelia, including those of breast ducts, pancreatic ducts, acinar cells of the prostate, sweat glands of skin, and glandular epithelium of the uterine cervix. Furthermore, AFM13 shows staining of some non-secretory epithelia, including those of the urinary bladder, the fallopian tube, the ureter, and epididymal epithelial cells in the testis. The relevance of these findings is unclear. However, the same pattern of epithelial binding has been observed in cynomolgus monkeys without adverse toxicity.

AFM13 clinical experience

A first-in-human (FIH) Phase I study with AFM13 was conducted and completed in patients with R/R HL (AFM13-101).¹⁶ Currently, there are three ongoing clinical studies with AFM13 in patients with CD30-positive lymphoid malignancies, namely, AFM13-102 (R/R T-cell lymphoma), AFM13-103 (R/R HL), and AFM13-201 (R/R HL). Data from the clinical studies AFM13-101 and AFM13-103 which informed this study design are provided below.

Phase I first-in-human clinical trial in relapsed/refractory HL

The clinical FIH study, AFM13-101, was conducted in patients with heavily pre-treated R/R HL.¹⁶ Escalating doses of AFM13 were administered to determine the safety and tolerability of AFM13. Twenty-eight patients (16 males, 12 females) were enrolled and received treatment with AFM13 in 8 dose cohorts. Twenty-four patients received increasing doses of AFM13 ranging from 0.01 to 7.0 mg/kg on a weekly dosing schedule for 4 weeks. In addition, 4 patients were treated with 4.5 mg/kg twice weekly for 4 weeks.

The median age was 38.5 years (range 19 to 72 years). Fourteen of the 28 patients (50%) had refractory disease and the remainder had relapsed disease; all were CD30-positive. Patients had received a median of 6 (range 3 to 11) previous lines of therapy for HL. Twenty-four patients (85.7%) had previously received radiotherapy and 22 patients (78.6%) had previously undergone stem cell transplantation (SCT). Nine patients had previously received BV.

All 28 patients were included in the Safety population, 2 patients had to be excluded from the Efficacy population: 1 patient withdrew the informed consent; 1 patient discontinued due to AEs of pneumonia and multi-organ failure. Twenty-three of the 28 patients experienced at least one AE which was evaluated as treatment-related by the investigator. Most AEs were mild or moderate; only 18 of 196 (9.2%) of documented AEs were NCI CTCAE Grade 3. One dose limiting toxicity (DLT) was documented; Grade 4 hemolytic anemia considered possibly related to AFM13. The patient subsequently died due to aspergillus pneumonia and multi-organ failure which was assessed as not/unlikely to be related to study drug. The most frequent adverse events were symptoms associated with infusion-related reactions including pyrexia, chills, headache, and nausea. Treatment-associated infections were rare; nasopharyngitis and pneumonia was documented in 5 and 4 patients, respectively.

After review of the safety data, the Independent Data Monitoring Committee (IDMC) concluded that the maximum feasible dose of 7 mg/kg following a weekly dose schedule was reached without toxicity concerns or reaching the MTD. Additionally, the 4 patients treated with 4.5 mg/kg twice weekly completed the treatment without toxicity concerns of the IDMC. Apart from the DLT event, administration of AFM13 was not associated with clinically significant changes in any laboratory parameters, nor was it associated with clinically significant changes in any vital signs or ECG parameters.

Twenty-six of 28 patients were eligible for efficacy evaluation. No patients had a complete response (CR) and 3 of 26 patients had a partial response (PR) at the final study visit. The ORR (CR + PR) was 11.5%. At the end of study assessment 13 patients (50%) had stable disease and 10 patients (38.5%) had disease progression.

Overall, the effect of AFM13 treatment was more pronounced in patient cohorts with AFM13 doses ≥ 1.5 mg/kg with an ORR of 23% (3/13).

Pharmacokinetic data were assessed across all dose cohorts. Geometric mean apparent terminal half-life of AFM13 was 8.72 to 19.2 h. In the range of doses at or above that which was considered minimally efficacious (≥ 1.5 mg/kg), the mean half-life was in the range of about 13-19 hours.

Over the entire dose range (0.01 to 7 mg/kg), systemic exposure to AFM13 increased in a slightly greater than dose proportional fashion; for a doubling in dose, systemic exposure to AFM13 was predicted to increase 2.14 and 2.30-fold for C_{max} and AUC_{0-∞}, respectively. For a dose increase of 1.5 mg/kg to 7 mg/kg (4.7-fold), geometric mean C_{max} and AUC_{0-∞} increased 9.6-fold and 13.2-fold, respectively (i.e. approximately 2- to 3-fold greater than expected based on a proportional relationship).

For pharmacodynamics, AFM13 treatment resulted in an increase of activated NK cells as measured by CD69 expression at their surface. The kinetics of activated NK cells was related to the PK profile i.e. the AFM13 serum concentration. All 28 patients in the study had measurable levels of sCD30 at the start of treatment. In 24 patients, the level was decreased at the end of treatment. All patients treated in dosing cohorts ≥ 1.5 mg/kg (n=13) had a significant decrease of sCD30 with complete depletion in the majority. Cytokine measurements in plasma did not reveal conclusive results.

In total, 9 patients had received BV; with 7 having received BV as the most recent prior treatment. In these 7 cases, all patients were refractory to BV. Of these 9 patients, 6 experienced a stabilization of disease on AFM13 and 3 had tumor shrinkage that did not meet the criteria for an objective response.

This study concluded that AFM13 is safe and well tolerated at weekly doses of up to 7 mg/kg, and 4.5 mg/kg dosed twice a week.

Rationale for dose selection

The rationale for the selected dose of 200 mg is based on the overall pre-clinical and clinical experience from the completed AFM13-101 Phase I study and the other ongoing AFM13 clinical studies, including consideration of the safety profile, PK,

pharmacodynamics and clinical antitumor activity in patients with R/R HL and CD30-positive T-cell lymphomas. In addition, an exposure-response analysis was completed based on the data from the AFM13-101 Phase I study.

Efficacy considerations

As mentioned above, the data from AFM13-101 suggested the AFM13 dose level of 1.5 mg/kg to be the clinically minimum effective dose. While the dose levels of 1.5 mg/kg and 4.5 mg/kg were similarly active for tumor reductions, only the 1.5 mg/kg cohort had patients who achieved an objective response.

Importantly, 1.5 mg/kg was also observed as the clinically minimum effective dose in AFM13-102 study where 2 of the 3 patients in the 1.5 mg/kg/week cohort and 2 of the 3 patients in 7 mg/kg/week CIV cohort achieved an objective response.

Safety considerations

AFM13 shows an acceptable safety profile with IRR as a known risk and infections as a potential risk (refer to Guidance for Investigator section of the Investigator's Brochure for more information). To mitigate the risk of developing IRRs due to AFM13 infusion, premedication with i.v. H1 and H2 antagonist and oral acetaminophen will be required prior to the infusion. A steroid premedication regimen was implemented in the monotherapy study AFM13-202, however, this is not considered for use in this combination study of AFM13 + CBNK cells as the lymphodepletion treatment seems to reduce IRRs based on the safety data of observed (no IRRs found in combination with CBNK cells of MDACC). In addition, all subjects who are treated with AFM13 must be observed for at least one hour post-infusion for signs of immunological reactions including IRR symptoms and symptoms of cytokine release (see section 8.7 for more details). Other potential risks for AFM13 are serious and opportunistic infections, for which there will be close monitoring with subjects receiving appropriate supportive care, as clinically indicated.

No cases of Cytokine Release Syndrome, ICANS or Graft versus Host Disease (GvHD) of any grade were reported.

PK considerations

While the PK data from the AFM13-101 showed that overall, the systemic exposure to AFM13 increased in a slightly greater than dose proportional fashion (an increase of 2.14 and 2.30-fold for C_{max} and AUC, respectively), the systemic exposure increase from the dose 1.5 mg/kg to 7 mg/kg (4.7-fold), geometric mean C_{max} and AUC increased 9.6-fold and 13.2-fold, respectively i.e. approximately 2- to 3-fold greater than expected based on a proportional relationship.

In addition, exposure-response analyses for the study showed that achieving an AUC_{0-∞} of at least 100,000 h*ng/mL appears to be important to increase the likelihood of achieving clinical benefit and the proposed stratified flat dose of 200 mg corresponded to a dose of approximately 3 mg/kg; which is also significantly lower than the dose of 7 mg/kg, a dose level that was associated with increased incidence and severity of AFM13-associated

IRRs as mentioned above.

Completed population PK analysis using data from AFM13-101 and AFM13-102, indicated there were three variables that had a statistically significant impact on PK parameters, as listed below:

- Dose: higher doses had a greater effect on the central volume of distribution (i.e. with higher doses, more of the drug stays within the central compartment)
- Body surface area (BSA): higher BSA had proportionately lower C_{max}; however, BSA had no significant impact on the AUC
- Albumin: lower albumin led to faster clearance, thereby impacting the AUC

These results do not alter the proposed dose selection of 200 mg; however, the effect of lower albumin will be further investigated by Affimed in a separate Phase II trial in patients with relapsed/refractory CD30-positive peripheral T-cell lymphoma or transformed mycosis fungoides. Based on the additional PK data from that planned study, Affimed will determine whether a higher dose of AFM13 is warranted for patients with very low albumin. Given the timeline for the Phase II trial, it is not expected that the AFM13 dose for the current trial will have to be adjusted. See Section 8.0. on AFM13 supply, storage and administration.

7.2 CLADRIBINE (Leustatin™)

Cladribine is a purine analog.

Mechanism of Action: Cladribine is a prodrug which is activated by phosphorylation and converted into the active moiety, Cd-ATP. This active form incorporates into DNA to result in the breakage of DNA strand and shutdown of DNA synthesis and repair. This also results in a depletion of nicotinamide adenine dinucleotide and adenosine triphosphate (ATP). Cladribine is cell-cycle nonspecific. The mechanism of cladribine in treating multiple sclerosis (MS) is unknown, but may involve cytotoxic effects on B and T lymphocytes that result from the shutdown of DNA synthesis, leading to a depletion of lymphocytes.

Known Side Effects: Neutropenia, thrombocytopenia, anemia, fever, infection (potentially severe; septicemia, pneumonia), acute renal insufficiency, nausea, vomiting, loss of appetite and diarrhea, headache, fatigue, rash and administration site reaction. Please refer to the package insert for a complete listing of all toxicities.

7.3 CYCLOPHOSPHAMIDE:

Therapeutic Classification: Antineoplastic alkylating agent

Pharmaceutical Data: Lyophilized cyclophosphamide for injection contains 75 mg of mannitol per 100 mg of cyclophosphamide (anhydrous) and is supplied in vials for single dose use.

Stability and Storage: Storage at or below 77°F (25°C) is recommended; this product will withstand brief exposure to temperatures up to 86°F (30°C) but should be protected from temperatures above 86°F. Reconstituted lyophilized cyclophosphamide for injection is chemically and physically stable for 24 hours at room temperature or for 6 days at 39°F (4°C); it does not contain any antimicrobial preservative and thus care must be taken to assure the sterility of prepared solutions.

Mechanism of Action: It interferes with DNA replication and transcription of RNA and ultimately results in the disruption of nucleic acid function.

Side effects: Dose limiting toxicity is hematologic. Nausea and vomiting are common. Anorexia, less frequently abdominal discomfort or pain and diarrhea may occur. There are isolated reports of hemorrhagic colitis, oral mucosal ulceration and jaundice occurring during therapy. Azotemia has been reported. Alopecia and hypersensitivity reactions can occur when high doses are utilized. Hemorrhagic cystitis may develop in patients treated with cyclophosphamide. Rarely this condition can be severe and even fatal. Acute cardiotoxicity has been reported with doses as low as 2.4 g/m. Interstitial pulmonary fibrosis has been reported in patients receiving high doses of cyclophosphamide over a prolonged period.

7.4 FLUDARABINE:

Therapeutic Classification: Antineoplastic agent; Antimetabolite (Purine Analog)

Pharmaceutical Data: Each vial contains 50 mg lyophilized drug, to be reconstituted with 2 ml sterile water to a solution that is 25 mg/ml for IV administration.

Solution preparation: Mix each vial with 2 ml sterile pyrogen-free water to a clear solution, which is 25 mg/ml for IV administration only. Reconstituted solution should be used within 8 hours.

Adverse effects:

Observed in more than 10%: Cardiovascular: Edema (8% to 19%); Central nervous system: Fever (11% to 69%), fatigue (10% to 38%), pain (5% to 22%), chills (11% to 19%); Dermatologic: Rash (4% to 15%); Gastrointestinal: Nausea/vomiting (1% to 36%), anorexia (34%), diarrhea (5% to 15%), gastrointestinal bleeding (3% to 13%); Genitourinary: Urinary tract infection (2% to 15%); Hematologic: Myelosuppression (nadir: 10-14 days; recovery: 5-7 weeks; dose-limiting toxicity), anemia (14% to 60%), neutropenia (grade 4: 37% to 59%; nadir: ~13 days), thrombocytopenia (17% to 55%; nadir: ~16 days); Neuromuscular & skeletal: Weakness (9% to 65%), myalgia (4% to 16%), paresthesia (4% to 12%); Ocular: Visual disturbance (3% to 15%); Respiratory: Cough (44%), pneumonia (3% to 22%), dyspnea (1% to 22%), upper respiratory infection (2% to 16%), rhinitis (11%); Miscellaneous: Infection (12% to 44%), diaphoresis (14%)

Observed in 1% to 10%: Cardiovascular: Peripheral edema (less than 7%), angina (less than 6%), chest pain (less than 5%), CHF (3%), arrhythmia (3%), cerebrovascular accident (3%), myocardial infarction (3%), supraventricular tachycardia (3%), deep vein

thrombosis (1% to 3%), phlebitis (1% to 3%), aneurysm (less than 1%), transient ischemic attack (1%); Central nervous system: Headache (9%), malaise (6% to 8%), sleep disorder (1% to 3%), cerebellar syndrome (less than 1%), depression (1%), mentation impaired (1%); Dermatologic: Alopecia (3%), pruritus (1% to 3%); Endocrine & metabolic: Hyperglycemia (1% to 6%), LDH increased (less than 6%), dehydration (1%); Gastrointestinal: Abdominal pain (10%), stomatitis (9%), weight loss (6%), esophagitis (3%), constipation (1% to 3%), mucositis (2%), dysphagia (1%); Genitourinary: Dysuria (3% to 4%), hesitancy (3%); Hematologic: Hemorrhage (1%), myelodysplastic syndrome/acute myeloid leukemia (usually associated with prior or concurrent treatment with other anticancer agents); Hepatic: Cholelithiasis (3%), liver function tests abnormal (1% to 3%), liver failure (1%); Neuromuscular & skeletal: Back pain (9%), osteoporosis (2%), arthralgia (1%); Auditory: Hearing loss (2% to 6%); Renal: Hematuria (2% to 3%), renal failure (1%), renal function test abnormal (1%), proteinuria (1%); Respiratory: Bronchitis (9%), pharyngitis (9%), allergic pneumonitis (6%), hemoptysis (1% to 6%), sinusitis (5%), epistaxis (1%), hypoxia (1%); Miscellaneous: Flu-like syndrome (5% to 8%), herpes simplex infection (8%), anaphylaxis (1%), tumor lysis syndrome (1%).

Observed in <1%, postmarketing, and/or case reports: Acute respiratory distress syndrome, agitation, blindness, blurred vision, bone marrow fibrosis, coma, confusion, diplopia, eosinophilia, Epstein-Barr virus (EBV) associated lymphoproliferation, EBV reactivation, erythema multiforme, Evans syndrome, flank pain, hemolytic anemia (autoimmune), hemophilia (acquired), hemorrhagic cystitis, herpes zoster reactivation, hyperkalemia, hyperphosphatemia, hyperuricemia, hypocalcemia, interstitial pneumonitis, metabolic acidosis, opportunistic infection, optic neuritis, optic neuropathy, pancreatic enzymes abnormal, pancytopenia, pemphigus, pericardial effusion, peripheral neuropathy, photophobia (primarily with high doses), progressive multifocal leukoencephalopathy (PML), pulmonary fibrosis, pulmonary hemorrhage, pulmonary infiltrate, respiratory distress, respiratory failure, Richter's syndrome, seizure, skin cancer (new onset or exacerbation), Stevens-Johnson syndrome, thrombocytopenia (autoimmune), thrombocytopenic purpura (autoimmune), toxic epidermal necrolysis, trilineage bone marrow aplasia, trilineage bone marrow hypoplasia, urate crystalluria, wrist drop.

Also observed: Neurologic syndrome characterized by cortical blindness, coma, and paralysis [36% at doses $>96 \text{ mg/m}^2$ for 5-7 days; $<0.2\%$ at doses $<125 \text{ mg/m}^2/\text{cycle}$ (onset of neurologic symptoms may be delayed for 3-4 weeks)].

Mechanism of Action: Fludarabine inhibits DNA synthesis by inhibition of DNA polymerase and ribonucleotide reductase; also inhibits DNA primase and DNA ligase I.

Human Safety and Pharmacology.

Distribution: Vd: $38\text{-}96 \text{ L/m}^2$; widely with extensive tissue binding

Protein binding: 2-fluoro-ara-A: ~19% to 29%

Metabolism: I.V.: Fludarabine phosphate is rapidly dephosphorylated in the plasma to 2-fluoro-ara-A (active metabolite), which subsequently enters tumor cells and is phosphorylated by deoxycytidine kinase to the active triphosphate derivative (2-fluoro-ara-ATP)

Bioavailability: Oral: 2-fluoro-ara-A: 50% to

65% **Half-life elimination:** 2-fluoro-ara-A: ~20 hours
Time to peak, plasma: Oral: 1-2 hours
Excretion: Urine (60%, 23% as 2-fluoro-ara-A) within 24 hours

Dose adjustment in renal and hepatic impairment: It appears that no adjustment is needed in hepatic impairment. Renal impairment dosing is **NOT** specific to stem cell transplant patients. In patients not-receiving a stem cell transplant, doses are typically reduced by 20% for CrCl of 30-70 ml/min and not used for CrCl < 30 ml/min.

No dose adjustments for renal and/or hepatic impairment related to fludarabine will be made in this study.

Monitoring Parameters: CBC with differential, platelet count, AST, ALT, serum creatinine, serum albumin, uric acid; monitor for signs of infection and neurotoxicity

8.0 Study Medication and Administration

8.1. Supply of AFM13.

Affimed will supply AFM13 study drug as a sterile lyophilized powder for reconstitution for intravenous (iv) infusion (**Table 4**). Full details on the preparation and administration of AFM13 are provided in the Investigator's Brochure.

The Investigator shall take responsibility for the study drug and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of study drug in accordance with the protocol and any applicable laws and regulations.

The Quality Control Standards and requirements for AFM13 study drug are described in separate release protocols/Certificate of Analysis.

Table 4: Composition of AFM13 for IV infusion

Active components:	One vial contains 10 mg lyophilized AFM13 for IV administration.
Excipients:	Polysorbate 20 0.01% (w/v) Trehalose dihydrate 8.0% (w/v) Sodium dihydrogen phosphate dihydrate 13.5 mM Di-Sodium hydrogen phosphate dihydrate 1.5 mM
Solvent/Reconstitution:	For reconstitution 7.5 mL Water for Injection per vial are required.
Stability:	Stability testing of AFM13 revealed a shelf life of 48 months.
Storage and handling:	The investigational medicinal product AFM13 is stored at 2-8°C (36-46°F). No special storage or handling instructions are required. The freshly prepared infusion solution may be kept at 2-8°C (36-46°F) for up to 24 hours prior infusion. This includes the transport time from the pharmacy to the site of administration. In case of cold storage, a warm up time of at least 30 minutes should be allowed before infusion. The infusion solution is stable for up to 24 hours at room temperature ($\leq 25^{\circ}\text{C}$ / $\leq 77^{\circ}\text{F}$), thus the accumulated time for warm up and infusion must not exceed 24 hours. Avoid direct exposure to sunlight during preparation and storage of the infusion solution.

8.2. Provision and Replacement of AFM13

Sufficient doses of AFM13 study drug will be supplied. Where study drug supplies (or packaging) are apparently damaged on receipt or considered unfit for use by the study site, the Sponsor (or their delegate) must be notified immediately. Where required, clinical trial supplies will be replaced. Further details on the handling of AFM13 study drug at site will be described in the Investigator's Brochure.

8.3. Labelling of AFM13

AFM13 clinical trial supplies will be labeled in compliance with Good Manufacturing Practice Annex 13 requirements, US Food and Drug Administration (FDA) requirements, and any other applicable local regulatory guidelines.

8.4. Storage of AFM13

AFM13 study drug will be shipped to the site and must be stored at the site in a secure location under controlled conditions and in the required temperature range (**Table 4**).

8.5. Drug Accountability

The Investigator is obliged to keep sufficient documentation of the delivery, use and destruction or return of unused, used or partially used AFM13 study drug. The

documentation must include dates, quantities, patient numbers, batch numbers or other identification number. The Investigator may assign some or all of the Investigator's duties for drug accountability to an appropriate pharmacist.

The Investigator should maintain records that document adequately that the patients were administered the doses specified in the protocol and reconcile all AFM13 study drug received for the trial.

AFM13 provided for this study is for use only as directed in the protocol. It is the Investigator and their institution's responsibility to establish a system for handling study drug so as to ensure that:

- deliveries of AFM13 are correctly received by a responsible person;
- such deliveries are recorded;
- study drug is handled and stored safely and properly as stated on the label;
- study drug is only dispensed to study patients in accordance with the protocol;
- and any unused study drug is destroyed locally according to MD Anderson Investigational Drug policy.

Certificates of delivery and return must be signed by the responsible pharmacist, and copies retained in the Pharmacy File. Throughout the study, it must be possible to reconcile delivery records with records of usage and any destroyed/returned stock of AFM13. To help with compliance checks, records of usage should include an appropriate form of identification of the patient to whom the study treatment was dispensed (using an indirect form to allow cross reference to the patients' identity), plus the quantity and date of dispensing.

8.6. Required Premedication Regimen and Post-dose Observation

Patients must receive premedication with the following medications before each dose of AFM13:

- iv H1/H2 antagonist e.g. diphenhydramine 50 mg or equivalent
- oral ibuprofen 400 mg or equivalent (if contraindicated, may substitute with acetaminophen 650 mg).

Patients must be observed for at least 1 hour following each AFM13 dose.

8.7. AFM13 Dosing Instructions for i.v. administration

AFM13 will be administered at a dose of 200 mg. All doses of AFM13 will be infused over 4 hours $\pm$ 15 minutes. Patients must be observed during the infusion and for at least 1 hour following each AFM13 dose for signs of immunological reactions including IRR symptoms and symptoms of cytokine release. Clinical symptoms include, but are not limited to: skin rashes, pruritus, urticarial, flu-like symptoms (fever, chills, muscle aches, headache), nausea, vomiting, diarrhea, low blood pressure, increased heart rate. For patients who experience such AEs during an AFM13 infusion, the infusion

rate may be slowed based on the instructions listed in Table 4.

8.8. AFM13 Dose Delays or Interruptions

The minimum time period between any two consecutive AFM13 administrations cannot be less than 3 days. In the case of an IRR or other AE which occurs during an AFM13 infusion, dose modifications, interruptions or delays may be utilized according to the guidance described in **Table 5 below**. Dose delays of up to 2 weeks (·14 days) are allowed for patients to recover from any AE and return to baseline or ·Grade 2. Patients should re-commence study therapy within 2 weeks of the delay. IRR has been the most frequently observed AE to date across all AFM13 studies (range 14-80%), as a single agent and in combination with pembrolizumab. In addition, treatment-related fever, chills and nausea, symptoms commonly associated with IRR, have also been reported frequently (range 23-54% across all studies). All patients on treatment with AFM13 should be closely monitored for these signs and symptoms.

Table 5: Management of AFM13 associated infusion-related reactions and other adverse events

CTCAE Grade	Management of reaction	Current AFM13 dose	Any subsequent AFM13 doses
Mandatory premedication regimen with H1 and H2 antagonist and NSAID (or acetaminophen)			
1	Treat per institutional guidelines based on symptomology.	No change. Consider reducing the infusion rate from 4 to 5 hours.	Consider reducing infusion rate from 4 to 5 hours for all subsequent AFM13 infusions, especially if Grade 1 IRR reoccurs. Or infusion rate will be per PI discretion
2	Treat per institutional guidelines based on symptomology.	Stop infusion until symptoms resolved. 1st occurrence: continue infusion on same day at reduced rate of 5 hours. 2nd occurrence: reduce AFM13 dose to 100 mg If the IRR reoccurs during the resumed infusion with the reduced 5 hour rate, or the reduced dose, omit the day's dose.	Reduce the infusion rate from 4 to 5 hours for all subsequent infusions. If the patient was dose rate or dose reduced at the previous infusion, maintain the reduced rate of 5 hours and the reduced dose of 100 mg for all subsequent infusions. Or infusion rate will be per PI discretion
3	Treat per institutional guidelines based on symptomology.	Stop and omit the rest of infusion for the day.	1st occurrence: reduce the infusion rate to 5 hours. Or infusion rate will be per PI discretion

CTCAE Grade	Management of reaction	Current AFM13 dose	Any subsequent AFM13 doses
Mandatory premedication regimen with H1 and H2 antagonist and NSAID (or acetaminophen)			
			2nd occurrence: reduce AFM13 dose to 100 mg If the patient was dose rate or dose reduced at the previous infusion, maintain the reduced rate of 5 hours and the reduced dose of 100 mg for all subsequent infusions. If Grade 3 IRR reoccurs despite the rate and dose reductions, the Sponsor must be consulted and discontinuation of AFM13 will be considered.
4	Consider transfer to intensive care unit. Treat per institutional guidelines based on symptomology.	Permanently stop AFM13 treatment.	

Abbreviations: CTCAE = common terminology criteria for adverse events; IRR = infusion-related reaction; NSAID = nonsteroidal anti-inflammatory drug.

9.0 Criteria for Removal from the Study

1. Patient withdrawal of the informed consent/authorization, or refusal to continue on study
2. Occurrence of an unacceptable toxicity (see section 8.8)
3. Inability to infuse AFM13-NK cell product due to product manufacturing issues
4. Not able to receive AFM13-NK cell infusion due to toxicity (see section 8.8)
5. Non-compliance with study procedures and monitoring
6. Lost to follow-up
7. Disease progression
8. Study completion at one year
9. Death
10. If for any reason, the PI feels it in the best interest of the patient to come off study

10.0 Statistical Considerations

This is a Phase I-II trial using the Bayesian Optimal Interval (BOIN) design [22] to determine the MTD of CB-NK cells preloaded with AFM13 (i.e., AFM13-NK) in patients with biopsy-positive CD30 who are undergoing stem cell transplant. Three levels of AFM13-NK will be studied as listed in Table 2. The BOIN design is implemented in a way similar to the traditional 3+3 design, but is more flexible and possesses superior operating characteristics that are comparable to those of the more complex model-based designs, such as the Continual Reassessment Method (CRM).[23]

Dose-limiting toxicity (DLT) will be defined as any related grade 3 or greater non-hematologic toxicity that occurs within 28 days of AFM13-NK (Cycle 1). The target toxicity rate $\phi = 0.30$. The choice of MTD will be guided only by data collected in Cycle 1. Patients who receive Cycle 2 will receive the same dose as received in Cycle 1. Doses that have not yet been studied in Cycle 1 will not be given in Cycle 2. Following the dose-escalation phase 1 the study will enter its phase 2 at its MTD, with a total enrollment of 46 patients. These will include 36 patients with HL and 10 patients with NHL.

Dose Escalation

In the dose-escalation portion, we will enroll and treat patients in cohorts of size 3. Three dose levels will be studied, starting at the lowest level. To guide dose-escalation decisions, if the DLT rate of the current dose cohort is ≤ 0.236 , the next cohort of patients will be treated at the next higher dose level; if it is ≥ 0.359 , the next cohort of patients will be treated at the next lower dose level. These boundaries were created when minimizing decision errors such that $\phi_1 = 0.18$ is the highest toxicity probability that is considered sub-therapeutic (underdosing) and $\phi_2 = 0.42$ is the lowest toxicity probability that is deemed overly toxic (overdosing). For the purpose of overdose control, doses will be eliminated from further examination if $\Pr(p_j > 0.30 \mid \text{data}) > 0.95$.

Dose escalation will be conducted as follows:

1. Patients in the first cohort are treated at dose level 1.
2. If a new patient is ready to enroll in the trial and there are fewer than 3 patients enrolled in the current open cohort, the next patient enrolled should be assigned the current dose.
3. If a new patient is ready to enroll in the trial and the current open cohort has 3 patients, the determination of dose for the next cohort of 3 patients will proceed according to **Table 6**. The following should be noted.
 - a. If none of the actions (i.e., escalation, de-escalation or elimination) are triggered, the new cohort of patients will be treated at the current dose.
 - b. If the rule indicates “eliminate”, the current dose and all higher doses should be eliminated from the trial because evidence indicates these doses are excessively toxic.
 - i. The next cohort of patients should be assigned the next

- lowest dose.
- ii. If the lowest dose is eliminated, the trial should be terminated because all doses are excessively toxic; no dose should be selected as the MTD.
- c. If the rule indicates de-escalation and the current dose is the lowest dose, treat the new cohort of patients at the lowest dose unless the number of DLTs reaches the elimination boundary, at which point terminate the trial for safety.
- d. If the rule indicates escalation and the current dose is the highest dose, treat the new cohort of patients at the highest dose.
- 4. Dose escalation continues in this manner until
 - a. The trial is stopped because all doses are eliminated.

Table 7 shows the operating characteristics associated with this design.

The selection of the MTD is based upon isotonic regression, and is defined as the dose for which the isotonic estimate of the DLT rate is closest to the target DLT rate. If there are ties, the higher dose will be chosen as the MTD when the isotonic estimate is lower than the target DLT rate, and the lower dose will be chosen when the isotonic estimate is greater than or equal to the target toxicity rate.

The dose escalation rules and operating characteristics were calculated using the BOIN R package version 2.6.4 (<https://cran.r-project.org/web/packages/BOIN/index.html>). This software will also be used to select the MTD at the end of this trial.

Table 6. Escalation rules

Actions	Number of patients treated at the current dose							
	3	6	9	12	15	18	21	24
Escalate if the number of patients with at least 1 DLT is less than or equal to:	0	1	2	2	3	4	4	5
Deescalate if the number of patients with at least 1 DLT is greater than or equal to:	2	3	4	5	6	7	8	9
Eliminate if the number of patients with at least 1 DLT is greater than or equal to:	3	4	5	7	8	9	10	11

Table 7. Operating characteristics

	Dose level			Number of Patients	% Early Stopping
	1	2	3		
True DLT rate	0.05	0.10	0.15		
Selection %	0.3	4.3	95.4		0.0
# pts treated	3.7	4.6	15.7	24	
True DLT rate	0.20	0.30	0.40		
Selection %	26.3	46.3	24.1		3.3
# pts treated	9.8	8.8	4.9	23.5	
True DLT rate	0.10	0.30	0.50		
Selection %	17.7	66.6	15.2		0.5
# pts treated	7.5	11.6	4.8	23.9	
True DLT rate	0.40	0.50	0.60		
Selection %	46.8	6.5	0.3		46.4
# pts treated	14.4	3.00	0.4	17.8	

The BOIN design will be implemented by the clinic using the rules above, with consultation from the biostatistical collaborator as necessary.

In the first part of the study the recommended dose of NK cells in combination with AFM13 will be established. If no DLTs occur up to the highest cell dose of 1×10^8 after 6 patients, this dose will be defined as recommended phase 2 dose and the efficacy of this dose in patients with HL and CD30+ NHL will be assessed.

The aim is to recruit a total of 30 response evaluable patients with HL and 10 response evaluable patients with CD30+ NHL at this dose level. Patients from the dose escalation part of the study who are response evaluable and treated at the recommended phase 2 dose, will be counted towards the final number of patients.

Update August 2021: We have treated a total of 3 patients at the lowest dose and 3 patients at the middle dose with no toxicity. A total of 3 patients have been treated at the highest dose to date with no toxicity. We will treat another 3 patients at this dose for a total of 6, and if none of these has toxicity, we will change the rest of the Phase I design to be a Phase II dose expansion at that dose. The total accrual will be 46 patients, including 6 patients treated at doses below the MTD, 30 patients with Hodgkin patients treated at the MTD, and 10 patients will be non-Hodgkin patients treated at the MTD. All patients with HL treated at the MTD will be pooled for analysis purposes.

Data Analysis

Adverse events will be summarized by dose. The distributions of time-to-event outcomes, including overall survival (OS) and event-free survival (EFS), will be estimated using the Kaplan-Meier method.[24] Log-rank and Cox regression analyses will be used to model these times with demographic and disease covariates of interest. No imputation for missing values is planned. Overall response rate (ORR), CR, and PR rates will be determined by the ratio of responses, CRs, and PRs, respectively, over the number of patients with measurable lesions. Logistic regression will be used to

assess the association between ORR and disease and demographic covariates of interest. The duration of response will be measured from the time of initial response to disease relapse/progression. Donor AFM13-NK cells and immune reconstitution studies will be summarized with descriptive statistics.

The Investigator is responsible for completing a safety summary report, and submitting it to the IND Office Medical Affairs and Safety Group, for review and approval. This should be submitted after the first 3 evaluable patients, complete 1 cycle of study treatment, and every 3 evaluable patients, thereafter before changing dose levels. Once the MTD has been established, enrollment will proceed uninterrupted, even though safety summary reports will continue to be submitted every 3 patients.

A copy of the cohort summary should be placed in the Investigator's Regulatory Binder under "sponsor correspondence".

11.0 Reporting Requirements

Definitions

Active

Treatment

For the purpose of this study, the investigational component of the treatment plan is the infusion of ex vivo-expanded and incubated with AFM13-NK cells and subsequent systemic infusions of AFM13.

Active treatment period

Is the first day of infusion of AFM13-NK cells as per treatment plans outlined above, up to 30 days of the last day of infusion of AFM13-NK cells.

Follow-up period

Is defined from 31 days of infusion of AFM13-NK cells until 1 year of treatment completion. Thereafter patients will be followed as standard procedures for hematopoietic transplant and cellular therapy recipients including monitoring for relapse, survival and late events.

Adverse Event

An adverse event is the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after the first protocol intervention, even if the event is not considered to be related to study treatment. Medical conditions/diseases present before starting study therapy are only considered adverse events if they worsen after starting study treatment. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy.

Assessment of the Adverse Events Severity

The severity of the adverse events (AEs) will be graded according to the Common

Terminology Criteria v5.0 (CTCAE) from the first day of infusion of AFM13-NK cells and up to 30 days of the last treatment intervention will be reported according to MDACC policy. Events not included in the CTCAE chart will be scored as follows:

General grading:

Grade 1: Mild: discomfort present with no disruption of daily activity, no treatment required beyond prophylaxis.

Grade 2: Moderate: discomfort present with some disruption of daily activity, require treatment.

Grade 3: Severe: discomfort that interrupts normal daily activity, not responding to first line treatment.

Grade 4: Life threatening: discomfort that represents immediate risk of death

Causality Assessment

The investigational component of the treatment plan of this study is the infusion of AFM13-NK cells and AFM13 after lymphodepleting chemotherapy.

Therefore, events known to be caused by the NK cell infusion and its direct consequences will be assessed as definitely related when assessing the causality.

When the relationship of the adverse event cannot be ruled out between the NK cell infusion and the treatment plan, the event will be scored as probably or possible related.

Events known to be related to drugs used as part of the treatment plan as well as to drugs used as supportive treatment will be scored as unrelated to the NK cells infusion.

Causality will be determined as per the guidelines above and based on the expected toxicities listed below.

The Principal Investigator or physician designee will be the final arbiter in determining the causality assessment.

Data collection

Collection of adverse events will reflect the onset and resolution date and maximum grade. Intermittent events should be labeled as such and followed until resolution. If a patient is taken off study while an event still ongoing, this will be followed until resolution unless another therapy is initiated. Pre-existing medical conditions will be recorded only if an exacerbation occurs during the active treatment period. Events not to be considered adverse events in this study are those related to original disease or expected in the original disease. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms or are considered clinically significant.

Patients will be registered in MD Anderson's Clinical Trial Management System,

OnCore.

The MDACC Internal Adverse Event Reporting Form will be used for reporting to the IRB and the Investigational New Drug (IND) Office. Serious adverse events must be followed until clinical recovery 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 occurring after the 30-day time period that are related to the study treatment must be reported to the IRB and the IND Office. This includes the development of a secondary malignancy.

Hospitalizations for treatment of disease, or related to complications from treatment administered prior to AFM13-NK cell infusion will not be captured. Collection of adverse events (AE) will begin at the time of the AFM13-NK cell infusion and will reflect the onset and resolution date and maximum grade. Intermittent events should be labeled as such and followed until resolution. If a patient is taken off study while an event is still ongoing, this will be followed until resolution unless another therapy is initiated.

Concomitant medications

Patients treated on this protocol will require supportive care treatment (concomitant medications). These medications are considered standard of care and have no scientific contribution to the protocol; therefore no data will be captured on various medications needed or their side effects. However, lymphodepleting chemotherapy, antibiotics and scheduled non-supportive meds administered from the day of lymphodepleting regimen to 30 days post AFM13-NK cell infusion will be captured in the medical record.

REDCap will be used as the electronic case report form for this protocol. Protocol specific data and AEs will be entered into REDCap.

Severity of the adverse events (AEs)

All AEs will be collected in Redcap. All grades of AEs related to AFM13-NK cells and systemic AFM13 infusions will be reported. The intensity of adverse events (AE) will be assessed according to the Common Terminology Criteria v5.0 (CTCAE). Adverse events unrelated to the AFM13-NK cells and AFM13 infusions will not be reported.

AEs related to the NK cells infusion:

1. These expected events will be monitored during the first 24 hours post infusion:

- Fever
- Chills
- Decrease in blood pressure
- Rash
- Shortness of breath

2. Unexpected and related events:

- Grade 4 NK cell infusion-related toxicity
- Failure to engraft by D+28 or delayed engraftment
- Grades 3-5 allergic reactions related to AFM13-NK cell infusion

- Grade 3-5 organ toxicity (cardiac, dermatologic, gastrointestinal, hepatic, pulmonary, renal/genitourinary, or neurologic) not pre-existing or due to the underlying malignancy or due to preparative chemotherapy and occurring within 30 days (+3 days if necessary) post-transplant
- Grades 3-4 acute GVHD occurring within 45 days post-transplant, treatment-related death within 8 weeks of the study cell infusion

Serious Adverse Event reporting (SAE)

An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or the sponsor, 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, 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 the IND Sponsor, IND Office.

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 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.

Oncore will be utilized for safety reporting to the IND Office and MDACC IRB.

Serious adverse events will be captured from the time of the first protocol-specific intervention, until 30 days after the last dose of drug, 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 30-day time period that are related to the study treatment must be reported to the IND Office. 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 PI and the research team 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.

Affirmed Reporting Requirements for SAE's:

A copy of the submitted eSAE form will be sent to Affirmed at the same time it is submitted to the IND Office per MDACC IND reporting policy.

spm2 – safety projects & more

GmbH Aurum 05

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69493 Hirschberg a. d. Bergstraße, Germany Safety Fax: +49 621 - 5 70 59 71

Email: Affirmed-PHV@spm2-safety.com (preferred option)

IND Reporting

During the course of the study, the Sponsor may determine that certain safety reports are required to comply with regulations. The Sponsor is responsible for submission of such reports to their IND. The Investigator may receive a letter called an "IND Safety Report" from this study and/or other Affirmed sponsored studies (cross reports). These reports must be submitted to the IRB/EC as required.

Annual IND Report as well as any IND amendments will be provided to spm² at the time of the provision to the MDACC IRB and the FDA.

Study Monitoring

The MDA IND office will be responsible for the monitoring of the study. The progress of the study will be reviewed on a regular basis to ensure adequate and accurate data collections. At each study monitoring visit, the Investigator will make available all records pertaining to the study. In addition to the IND Office, due to institutional conflict of interest, the protocol will be monitored for safety and efficacy by the MD Anderson external DSMB annually from the date of WIRB approval.

MDA Recommended AE Recording guidelines

We will follow these guidelines (Table 8)

Table 8. MDA Recommended AE Recording guidelines

Adverse Event Recording Guidelines					
Attribution	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Unrelated	Phase I	Phase I	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III
Unlikely	Phase I	Phase I	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III
Possible	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III
Probable	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III
Definitive	Phase I Phase II	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III	Phase I Phase II Phase III

12.0 Appendix 1

HIGHLY EFFECTIVE CONTRACEPTION

Both sexually active females of childbearing potential and non-vasectomized male patients with female partners of childbearing potential are required to use highly effective methods of contraception, initiated 14 days prior to first dose of study drug, continued during study treatment, and for at least 60 days after the last dose of study drug. Note that for non-vasectomized male patients, some of these methods are applicable to their female partners.

Highly effective methods of contraception, including abstinence, are defined as follows:

- Combined (estrogen- and progestogen-containing) hormonal contraception associated with the inhibition of ovulation; may be oral, intravaginal or transdermal method;
- Progestogen-only hormonal contraception associated with the inhibition of ovulation; may be oral, injectable, implantable method;
- An intrauterine device;
- Intrauterine hormone-releasing system;
- Bilateral tubal occlusion;
- Vasectomized partner of a female patient (provided that the vasectomized partner is the sole sexual partner of the woman of childbearing potential study participant and that the vasectomized partner has received medical assessment of surgical success); *or*
- Sexual abstinence (defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment, starting prior to first dose of study drug, for the duration of study treatment, and for at least 60 days after the last dose of study drug. Total sexual abstinence should only be used as a contraceptive method if it is in line with a patient's usual and preferred lifestyle. Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of exposure to study drug, and withdrawal methods, are not acceptable methods of contraception.

Barrier contraception (including male and female condoms with or without spermicide) is not considered a highly effective method of contraception. If used, this method must be utilized in combination with another acceptable method listed above.

13.0 Appendix 2

LYRIC RESPONSE CRITERIA

Table 2.

CR	PR	PD
PET-CT, score 1, 2, or 3* with or without a residual mass on 5PS† OR on CT, target nodes/nodal masses must regress to ≤ 1.5 cm in LDi	PET-CT score 4 or 5 with reduced uptake compared with baseline and residual mass(es) of any size. OR On CT $\geq 50\%$ decrease in SPD of up to 6 target measurable nodes and extranodal sites	• PET-CT score 4 or 5 with an increase in intensity of uptake from baseline and/or new FDG-avid foci consistent with lymphoma at interim or end-of-treatment assessment. OR On CT, an individual node/lesion must be abnormal with: LDi > 1.5 cm and increase by $\geq 50\%$ from PPD nadir and an increase in LDi or SDi from nadir 0.5 cm for lesions ≤ 2 cm 1.0 cm for lesions > 2 cm • In the setting of splenomegaly, the splenic length must increase by $> 50\%$ of the extent of its prior increase beyond baseline (eg, a 15-cm spleen must increase to > 16 cm). If no prior splenomegaly, must increase by ≥ 2 cm from baseline. New or recurrent splenomegaly • New or clear progression of preexisting nonmeasured lesions • Regrowth of previously resolved lesions • A new node > 1.5 cm in any axis or a new extranodal site > 1.0 cm in any axis; if < 1.0 cm in any axis, its presence must be unequivocal and must be attributable to lymphoma • Assessable disease of any size unequivocally attributable to lymphoma AND/OR new or recurrent involvement of the bone marrow • IR(1): $\geq 50\%$ increase in SPD in first 12 weeks • IR(2): $< 50\%$ increase in SPD with a. New lesion(s), or b. $\geq 50\%$ increase in PPD of a lesion or set of lesions at any time during treatment • IR(3): Increase in FDG uptake without a concomitant increase in lesion size meeting criteria for PD

IR, immune response; LDi, longest diameter; PPD, product of the perpendicular diameters; SDi, short diameter; 5PS, 5-point scale.

14.0 Appendix 3

TIMELINE OF SEND-OUT RESEARCH LABS

1) PK sampling of AFM13 (patients in cohorts 3):

Cycle 1:

Day	Time	Sample #
0	Before infusion of AFM13-NK	1
	End of infusion	2
+1	AM	3
+2	AM	4
+7	Before infusion of AFM13	5
	End of infusion	6
+9	AM	7
+14	Before infusion of AFM13	8
	End of infusion	9
+21	Before infusion of AFM13	10
	End of infusion	11
+22	AM	12

Cycle 2:

Repeat all 12 samples if the patient receives cycle 2

Additional cycles (Cycle 3 and beyond)

Day	Time	Sample #
0	Before infusion of AFM13-NK	1

2) Antidrug antibodies (ADA) (patients in cohorts 3):

Cycle 1:

Day	Time	Sample #
0	Before infusion of AFM13-NK	1
+14	Before infusion of AFM13	2
+21	Before infusion of AFM13	3

Cycle 2:

Repeat all 3 samples if the patient receives cycle 2

Additional cycles (Cycle 3 and beyond)

Day	Time	Sample #
0	Before infusion of AFM13-NK	1

3) Serum CD30 (patients in cohorts 3 and 4):

Cycle 1:

Day	Time	Sample #
0	Before infusion of AFM13-NK	1
+1	AM	2
+21	Before infusion of AFM13	3
+22	AM	4

Cycle 2:

Repeat all 4 samples if the patient receives cycle 2

Additional cycles (Cycle 3 and beyond)

Day	Time	Sample #
0	Before infusion of AFM13-NK	1

15.0 References

1. Forero-Torres A, Leonard JP, Younes A, et al. A Phase II study of SGN-30 (anti-CD30 mAb) in Hodgkin lymphoma or systemic anaplastic large cell lymphoma. *Br J Haematol* 2009; 146:171-9.
2. Younes A, Gopal AK, Smith SE, et al. Results of a pivotal phase II study of brentuximab vedotin for patients with relapsed or refractory Hodgkin's lymphoma. *J Clin Oncol* 2012;30:2183-9.
3. Connors JM, Jurczak W, Straus DJ, et al. Brentuximab Vedotin with Chemotherapy for Stage III or IV Hodgkin's Lymphoma. *N Engl J Med* 2018;378:331-44
4. Moskowitz CH, Nademanee A, Masszi T, et al. Brentuximab vedotin as consolidation therapy after autologous stem-cell transplantation in patients with Hodgkin's lymphoma at risk of relapse or progression (AETHERA): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* 2015;385:1853-62.
5. Horwitz S, O'Connor OA, Pro B, et al. Brentuximab vedotin with chemotherapy for CD30-positive peripheral T-cell lymphoma (ECHELON-2): a global, double-blind, randomised, phase 3 trial. *Lancet* 2019;393:229-40.
6. Pro B, Advani R, Brice P, et al. Five-year results of brentuximab vedotin in patients with relapsed or refractory systemic anaplastic large cell lymphoma. *Blood* 2017;130:2709-2717.
7. Prince HM, Kim YH, Horwitz SM, et al. Brentuximab vedotin or physician's choice in CD30-positive cutaneous T-cell lymphoma (ALCANZA): an international, open-label, randomised, phase 3, multicentre trial. *Lancet* 2017;390:555-66.
8. Guillemy C, Smyth MJ: NK Cells and Cancer Immunoediting. *Curr Top Microbiol Immunol* 2016;395:115-45.
9. Long EO, Kim HS, Liu D, et al: Controlling natural killer cell responses: integration of signals for activation and inhibition. *Annu Rev Immunol* 2013;31:227-58.
10. Morvan MG, Lanier LL. NK cells and cancer: you can teach innate cells new tricks. *Nat Rev Cancer* 2016;16:7-19.
11. Poppema S. Immunobiology and pathophysiology of Hodgkin lymphomas. *Hematology Am Soc Hematol Educ Program* 2005;231-238.
12. Reisners KS, Kessler J, Sauer M, et al. Rescue of impaired NK cell activity in Hodgkin lymphoma with bispecific antibodies in vitro and in patients. *Mol*

Ther 2013;21:895-903.

13. Shi J, Tricot G, Szmania S, et al: Infusion of haplo-identical killer immunoglobulin-like receptor ligand mismatched NK cells for relapsed myeloma in the setting of autologous stem cell transplantation. *Br J Haematol* 2008;143:641-53.
14. Szmania S, Lapteva N, Garg T, et al: Ex vivo-expanded natural killer cells demonstrate robust proliferation in vivo in high-risk relapsed multiple myeloma patients. *J Immunother* 2015;38:24-36.
15. Shah N, Martin-Antonio B, Yang H, et al: Antigen presenting cell-mediated expansion of human umbilical cord blood yields log-scale expansion of natural killer cells with anti-myeloma activity. *PLoS One* 8:e76781, 2013.
16. Shah N, Li L, McCarty J, et al. Phase I study of cord blood-derived natural killer cells combined with autologous stem cell transplantation in multiple myeloma. *Br J Haematol* 2017;177(3):457-466.
17. Reusch U, Burkhardt C, Fucek I, et al. A novel tetravalent bispecific TandAb (CD30/CD16A) efficiently recruits NK cells for the lysis of CD30+ tumor cells. *MAbs* 2014;6:728-39.
18. Rothe A, Sasse S, Topp MS, et al. A phase 1 study of the bispecific anti-CD30/CD16A antibody construct AFM13 in patients with relapsed or refractory Hodgkin lymphoma. *Blood* 2015;125(26):4024-31.
19. Sawas A, Elgedawe H, Vlade George, et al. Clinical and biological evaluation of the novel CD30/CD16A tetravalent bispecific antibody AFM13 in relapsed or refractory CD30-positive lymphoma with cutaneous presentation: A biomarker phase 1b/IIa study (NCT03192202). Abstract 2908, presented at ASH2018.
20. Bartlett NL, Chen RW, Domingo E, et al. A phase 1b investigating the combination of the tetravalent bispecific NK cell engager AFM13 and pembrolizumab in patients with relapsed/refractory Hodgkin lymphoma after brentuximab vedotin failure: Updated safety and efficacy data. Abstract 1620, presented at ASH 2018.
21. Cheson BD, Ansell S, Schwartz L, et al. Refinement of the Lugano Classification lymphoma response criteria in the era of immunomodulatory therapy. *Blood* 2016;128(21):2489-96.
22. Liu S and Yuan Y. Bayesian Optimal Interval Designs for Phase I Clinical Trials. *JR Stat Soc Ser C* 2015; 64:507-523.
23. O'Quigley J, Pepe M and Fisher L. Continual Reassessment Method: A Practical Design for Phase 1 Clinical Trials in Cancer. *Biometrics* 1990;46(1):33-48.
24. Kaplan, EL and Meier, P: Nonparametric estimator from incomplete observations. *J. American Statistical Association* 53:457-481, 1958.