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CAR-T cell therapy for liver metastases

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Thesis

CAR-T CELL THERAPY FOR LIVER METASTASES

by

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Liver metastases are the most common cause of death in colorectal cancer patients. The standard of care and potential for cure for colorectal liver metastases is resection, but often times disease it too extensive for this treatment. Over the years, cancer research has made way for advances in treating progressive disease through immunotherapy. By genetically modifying an individual’s immune system using virally transduced chimeric antigen receptor T cells (CAR-T), patients are better able to receive exquisitely specific T cells to target specific tumors. Furthermore, selective delivery strategies may enhance efficacy while limiting detrimental, systemic adverse effects. Not only this, CAR-Ts have also lead to complete remission in some liquid tumors while maintaining the potential for remission in solid tumors as well. This literature review takes readers through the emergence of the different generations of CAR-T and the various studies including clinical trials that have demonstrated the safety and efficacy of CAR-T.

The second portion of this paper will outline the design for a phase II clinical trial using intrahepatic CAR-T therapy in addition to selective internal radiation therapy (SIRT) for refractory CEA+ colorectal liver metastases. Benefits and limitations of using these therapies are further discussed.
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LIST OF ABBREVIATIONS

ACT ................................................................. Adoptive Cell Therapy
ALL ................................................................ Acute Lymphoblastic Leukemia
APC .............................................................. Antigen presenting cell
AST .................................................................. Aspartate transaminase
BUMC IRB ...................... Boston University Medical Campus Institutional Review Board
CAIX ................................................................ Carbonic anhydrase IX
CAR ................................................................. Chimeric Antigen Receptor
CAR-T .................................................................. Chimeric Antigen Receptor T cells
CDR ............................................................. Complementarity determining region
CEA ................................................................ Carcinoembryonic antigen
CLL .................................................................. Chronic Lymphocytic Leukemia
CLM .................................................................. Colorectal Liver Metastases
CPT 11 ................................................................... Irinotecan
CRC .................................................................. Colorectal Cancer
C Region .......................................................... Constant region
CRS .......................................................... Clinical Risk Score/ Cytokine Release Syndrome
CTCAE .................................................... Common Terminology Criteria for Adverse Events
DFI ................................................................... Disease Free Interval
DFS ................................................................... Disease Free Survival
FISH .................................................................. Fluorescence in situ hybridization
FOLFIRI ......... Combination of Leucovorin (FOL), Fluorouracil (F), Irinotecan (IRI)
FOLFOX............Combination of Leucovorin (FOL), Fluorouracil (F), Oxaliplatin (OX)
FOLFOXIRI........Combination of Leucovorin (FOL), Fluorouracil (F), Oxaliplatin (OX), Irinotecan (IRI)
FU ................................................................................................Fluorouracil
GMP ...........................................................................Good Manufacturing Practice
GONO .................................................................Gruppo Oncologico Nord Ovest
HAI ........................................................................Hepatic artery infusion
HAIC .................................................................Hepatic-Arterial Infusional Chemotherapy
HCC ........................................................................Hepatocellular carcinoma
H&EO ...............................................................Hematoxylin and Eosin
HITM ...............................................................Hepatic Immunotherapy for Metastases
HLA ........................................................................Human Leukocyte Antigen
HORG ............................................................The Hellenic Oncology Research Group
H region ..................................................................Heavy region
IFNγ .................................................................Interferon gamma
IL-2/12 ................................................................Interleukin 2/12
IRB ........................................................................Institutional Review Board
ITAM ..........................................................Immunoreceptor tyrosine-based activation motif
LOHP ....................................................................Oxaliplatin
L region ................................................................Light region
LV .........................................................................Leucovorin/Folinic Acid
mAb ........................................................................Monoclonal antibody
MHC .......................................................... Major Histocompatibility Complex
mRECIST................................................ modified Response Evaluation Criteria in Solid Tumors
OS .............................................................................................................. Overall Survival
PBMC ..................................................................................................... Peripheral blood mononuclear cell
PFS ........................................................................................................ Progression Free Survival
RCC ........................................................................................................... Renal Cell Carcinoma
scFv ......................................................................................................... Single-chain variable fragment
SIRFLOX .......................................................... Selective Internal Radiation (SIR) + FOLFOX
SIRT ........................................................................................................ Selective Internal Radiation Therapy
TCR ........................................................................................................... T cell receptor
TIL ........................................................................................................... Tumor-infiltrating lymphocyte
TLS ........................................................................................................... Tumor lysis syndrome
TRUCK .............................................................. T cells redirected against universal cytokine-mediated killing
V Region .................................................................................................. Variable region
WIRB ..................................................................................................... Western Institutional Review Board
INTRODUCTION

Background

Colorectal cancer (CRC) is one of the most prevalent cancers in the United States, more specifically the 4th most common cancer and the second leading cause of cancer-related deaths.\(^1\) Risk factors include advanced age, male sex, and African American descent. According to the National Cancer Institute, there were approximately 1,177,556 people living with CRC in the U.S. in 2013. The overall 5-year survival rate is 65%; if the cancer remains localized, 5-year survival is roughly 90%. However, once metastatic or stage IV disease is diagnosed, likelihood of survival at 5 years plummets to a mere 13%.\(^1\) One of the major manifestations of CRC is metastasis to the liver, which occurs roughly in 30% of cases at the time of presentation, and an additional 30% at some point during the course of their disease. Liver metastasis is a major cause of death in patients with primary adenocarcinoma; only 20% of patients with colorectal liver metastases (CLM) have resectable disease while the other 80% will most likely die despite receiving several lines of chemotherapy.\(^2\)

Cancer researchers have recently made several breakthroughs in developing alternatives to traditional therapy, which includes surgical resection, chemotherapy, and/or radiation therapy. These novel approaches range from cancer vaccines to adoptive immunotherapy using genetically modified chimeric antigen receptor T cells (CAR-T). CAR-T therapy has been utilized in the past few years and has shown promise in either stabilizing disease or mediating tumor regression in cases of lymphoma or leukemia, so called “liquid tumors.”\(^3,4\) The difficulty, however, has been in treating solid tumors due to
challenges in delivering a sufficient number of CAR-T while sparing normal organs. Current research has been attempting to overcome these boundaries to make CAR-T therapy safe and efficient in treating solid tumors as well.

**Statement of the Problem**

The standard of care for CLM, which is also potentially curative, is currently resection in the context of multidisciplinary management. However, what happens when disease is so extensive that resection is not an option and multiple chemotherapy trials have failed? CAR-T immunotherapy is one of the most recent, innovative alternative therapies to cancer treatment. While research has shown that CAR-T therapy has been effective in eradicating blood-borne cancers and lymphomas, it is more difficult to assess how they would fare in patients with progressive CLM. This study addresses the following question: would CAR-T infusions plus radiation therapy be safe and effective in chemotherapy-resistant and progressing liver metastases originating from gastrointestinal adenocarcinoma?

**Hypothesis**

Regional hepatic infusion of CAR-T in combination with selective internal radiation therapy (SIRT) is safe and effective for treating advanced, unresectable carcinoembryogenic antigen (CEA) + liver metastases that are refractory to other interventions. Safety will be measured using the Common Terminology Criteria for Adverse Events (CTCAE) Protocol. The efficacy of this intervention combination will be
assessed in the form of progression-free survival (PFS) by grading tumors from imaging data via the modified Response Evaluation Criteria in Solid Tumors (mRECIST).

**Objectives**

The cure for cancer has always been a relevant topic in the scientific research realm and an important societal goal. However, understanding that cancer is not one disease, but a highly heterogeneous group of conditions, make a personalized therapy such as CAR-T appealing. This therapy is generated in such a way to target an individual’s specific malignancy based upon validated bioassays. There is a current standard of care for CLM that has lead to prolonged disease free-survival in many patients or cure in those eligible for complete surgical resection. However, these treatments are not without adverse events. Chemotherapy uses highly toxic drugs with variable side effects while resection is associated with the risks related to major abdominal surgical procedures. If disease is too extensive, as is the case in most CLM patients, resection may not even be possible. The use of regional immunotherapy attempts to decrease the risk of systemic adverse events while also being more effective compared to the previous modalities. It has already shown to be safe in patients with CLM, for example. Radiation therapy also has a number of adverse events that correlate with higher incidence if given more frequently or over a larger surface area. This study further explores the safety and efficacy of using CAR-T plus a single administration of radiation therapy and how re-engineering of patient T cells may effectively treat liver metastases.
Specific Aims

- To assess the safety and efficacy of using CAR-T cell liver infusions in combination with radiation therapy by conducting a Phase II clinical trial.
- Assess progression-free survival in this population of patients as the primary outcome.
REVIEW OF THE LITERATURE

Overview

Colorectal cancer (CRC) causes close to 500,000 deaths worldwide annually. The majority of these deaths are attributed to metastatic disease to the liver, as mentioned above. Once CRC has transitioned to late stage metastatic disease, it is extremely difficult to stabilize, let alone to cure. This review of the literature will take readers through the current standard of treatment for CLM, the use and efficacy of immunotherapy, specifically CAR-T therapy in various liquid and solid tumors, immunosuppression in the liver and reasons why new approaches must be explored to treat liver disease, and finally, current research where CAR-T therapy has been used in CLM.

Existing research

Current standard of care for CLM

Over the past 20 years, the standard of care for CLM has been resection. This has been the only therapy that has been shown to be curative. However, some have criticized resection as the gold standard due to a lack of prospective randomized clinical trials assessing this data. However legitimate these concerns, it may not be completely feasible for surgeons to continually evaluate the benefits of resection for long periods of time and instead they base their reasoning to resect on retrospective data that has already been established. Importantly, it is undeniable that 15-20% of patients with CLM are cured following liver resection. In addition, when thinking of metastasis related to any malignancy, most would consider this a state of systemic disease. When CRC
metastasizes, it metastasizes to the liver 60-70% of the time\textsuperscript{6,8} and out of those patients, 20-35% will not have evidence of any extra-hepatic involvement. Many patients with multifocal metastases will have liver-dominant disease rendering liver-directed therapy an important component of their management. Although the disease has still metastasized from the distal GI tract, it is confined to the liver and thus, resection is a possible means of cure for CLM and the only curable therapy thus far. CAR-T may very well be the first step in treating CLM in order to possibly convert to resectability.

Tomlinson et al. performed a retrospective review looking at long-term disease-free survival from a hepatobiliary database of patients with CLM at least 10 years post-resection.\textsuperscript{6} Their aims were to assess whether resection was a true cure for CLM and whether other characteristics of disease including tumor burden were major prognostic factors. The study included 644 patients from Memorial Sloan-Kettering Cancer Center with CLM who had their tumors resected between the years 1985-1994. This study design afforded robust follow-up in order to assure more accurate survival data. They looked at a number of variables including age, sex, disease-free interval (DFI), which was characterized by the time period from primary resection to recurrence, size or number of CLMs, the extent of resection, pre-resection carcinoembryonic antigen (CEA) levels, and the necessity for adjuvant hepatic-arterial infusional chemotherapy (HAIC). The clinical risk score (CRS) was used to predict survival. This number takes into account several preoperative factors including DFI < 12 months, presence of multiple hepatic metastases, (the largest one being > 5 cm), and CEA > 200 ng/mL. Each factor was given a value of 1 point. A score of 5 was equivalent to a worse prognosis. This score as well as Kaplan-
Meyer survival curves were used to assess whether resection could lead to long-term survival and can thus be considered a cure for CLM.\textsuperscript{6}

Results indicated that out of all the patients assessed, 102 were found to be cancer survivors 10 years from the time of resection without any evidence of recurrent disease.\textsuperscript{6} The Kaplan-Meier survival curve in Figure 1 shows a plateau beginning at 10 years depicting the 102 patients who had actual disease-free survival. This represents a 17-25\% cure rate. Out of these 102 patients, 16 had recurrent disease, which was treated with another surgical resection; only one of those 16 patients died a cancer-related death. Nevertheless, researchers were able to define cure at 10 years post resection.\textsuperscript{6}

![Figure 1: 10-year survival in CLM patients post resection; Kaplan-Meier plot of disease-specific survival for 612 patients with potential 10-year follow-up who underwent resection of colorectal liver metastases from 1985 to 1994 at Memorial Sloan-Kettering Cancer Center (adapted from Tomlinson et al; Actual 10-Year Survival After Resection of Colorectal Liver Metastases Defines Cure).\textsuperscript{6}](image)

While resection is potentially curative, what happens when the extent of disease is so great that surgical resection is not an option? Data show that a mere 10-15\% of people with CLM are actual candidates to receive resection with a 5-year survival of 35\%.\textsuperscript{9,2} In
this situation, patients could be treated with neoadjuvant systemic or hepatic artery infusion (HAI) chemotherapy, or a combination of both in order to convert their disease to resectability. Conversion is even more likely if the extent of disease remains confined to the liver. It has been shown in a previous study that using a combination of fluorouracil, leucovorin, and oxaliplatin lead to a 15% increase in conversion to resectability for CLM. Using HAI would decrease the need for high amounts of systemic chemotherapy and thus decrease systemic toxic effects.²

A phase I clinical trial was conducted involving 49 patients with unresectable liver metastases from primary colorectal adenocarcinoma.² Characteristics of these patients’ disease included the following: involvement of 6 or more segments, major vessel involvement, and absence of disease outside the liver. Disease in both lobes and number of liver tumors were not factors excluding patients from resection but adequate liver functioning was required in order to enter trial. A CT angiogram was performed on all patients in order to assess blood supply and possible tumor involvement of the hepatic arteries. Patients then underwent pump surgery where the infusional pump catheter was placed in the hepatic circulation approximately 2-3 weeks before they were divided into two treatment cohorts; the first cohort received 4 weeks of systemic chemotherapy (oxaliplatin and irinotecan) while concurrently receiving HAIs of floxuridine and dexamethasone. The second cohort received 5 weeks of HAI therapy (floxuridine and dexamethasone) with escalating doses of systemic chemotherapy (oxaliplatin and irinotecan). Measured outcomes included tumor regression, disease progression, or extreme toxicity.²
The major toxicities that patients experienced in both cohorts were grade 3-4 diarrhea and neutropenia. Later toxicities include neurotoxicity, elevated bilirubin, elevated alkaline phosphatase and aspartate transaminase (AST). In regards to clinical activity, results showed that out of the total sample population of 49 patients, 45 patients (92%) had either a complete (8%) or partial response (84%). A complete response was characterized by absence of any disease on CT and normal CEA levels while a partial response was characterized by a 50% or greater reduction in tumor size. Lastly, stable disease, defined as a reduction in tumor size, was less than 50% in the absence of progression. Researchers also found that for those patients who had never received chemotherapy in the past, 100% of them responded to this treatment regimen. Twenty-three of these patients were able to undergo resection, followed by administration of systemic chemotherapy. Interestingly enough, extent of disease in these patients did not correlate with a decreased probability of resection. Resectability was not related to number or size of metastases, clinical risk score, primary tumor location, or involvement of the major vessels surrounding the liver, as determined by univariate analysis. Several patients who had involvement of all 8 liver segments as well as total hepatic vein involvement were candidates for resection at the end of the trial. In fact, the only variable that was associated with increased conversion to resectability was female sex, with an 81% and 30% resection rate for females and males respectively (P=.006). When comparing the two different cohort treatments, the resection rate for the 4-week treatment regimen was 36% and for the 5-week regimen, 56%.2
In conclusion, Kemeny et al. found that in order for extensive disease to become resectable, it must first be down-staged.\(^2\) In addition, certain characteristics of “advanced” disease such as tumor size and number do not preclude downstaging of CLM to resectable status. The researchers state that one of the greatest discrepancies that prevents other researchers from gathering accurate data that links adjuvant therapy with resectability is non-uniform criteria when comparing different trials. The definition of “unresectable disease” and delineation of the factors that would lead to resectability is not always the same across the board, thus making it difficult to determine whether disease is truly resectable, and thus might be eradicated sooner with the appropriate intervention.\(^2\)

Systemic Chemotherapy in CLM

The first-line chemotherapy regimen that results in the best outcomes in overall survival for advanced CRC or unresectable CLM has been exposure to the following cytotoxic agents: Fluorouracil (FU) + leuvocorin (LV), Irinotecan (CPT-11), and Oxaliplatin (LOHP).\(^{10}\) This combination is also known as FOLFOXIRI. Another combination of Fluorouracil, Leucovorin, and Irinotecan (FOLFIRI) has also shown to be efficacious; this was the first-line therapy before FOLFOXIRI emerged. The Hellenic Oncology Research Group (HORG) ran a multicenter randomized Phase III clinical trial comparing FOLFOXIRI and FOLFIRI.\(^{11}\) However, they did not find any significant difference in overall survival (\(P=0.337\)), time to disease progression (\(P=0.17\)), and response rates (\(P=0.168\)) between the FOLFOXIRI and FOLFIRI arms. They did find higher rates of alopecia, diarrhea, and neurotoxicity in the FOLFOXIRI group.\(^{11}\)
Another group of oncologists (The Gruppo Oncologico Nord Ovest, GONO) conducted another phase III trial comparing these two chemotherapy regimens in order to assess PFS and overall survival (OS). They randomly assigned 244 patients with unresectable, chemotherapy-naïve CLM to the two different treatment arms. The FOLFOXIRI patients had developed neurotoxicities and neutropenia, but they generally tolerated the treatment well. This group had an increased rate of secondary resection post therapy, a 3-month increase in PFS, longer OS and decreased early progression to disease.

As previously mentioned, chemotherapy is often used in order to convert disease to resectability but it is also used with adjuvant intent after resection. This is because recurrence of disease (either hepatic or disease outside the liver) develops in 70% of patients who have undergone resection. Half of those recurrences occur in the liver within the first two years after resection. Nordlinger et al. conducted a Phase III trial assigning 364 patients with resectable colorectal liver metastases into two treatment arms. Patients in the first arm received perioperative FOLFOX-4 and resection while the second arm received surgery alone. The reasoning behind giving perioperative chemotherapy was to treat micrometastatic disease and to ensure administration of systemic therapy. Results showed an increase in PFS in the first arm (P=.058) but no overall significant differences in OS of the two arms. However, the patients treated with FOLFOX also developed higher levels of postoperative complications including hepatic failure, intra-abdominal infection, and biliary fistula.
While chemotherapy may have a good response rate and may be used in various ways to allow either for resectability in some patients or perhaps increased overall survival post-resection, the cure rate of chemotherapy alone is still currently negligible. Recurrence may still occur even after adjuvant chemotherapy.

**Immunotherapy**

The immune system has been studied extensively and yet researchers are always discovering something new about it. This has been especially true in the past several years; our study and knowledge about tumorigenesis and how tumors evade the immune system has lead to the development of new cancer therapies. The typical treatment for any type of malignancy has traditionally been surgery, chemotherapy, and/or radiation therapy. However, there is an urgent need for therapies capable of offering cures to the large number of patients with metastatic solid tumors. A few years ago, cancer therapy has benefited from several breakthroughs in new alternatives to the typical regimens. Immunotherapy has been gaining more and more attention due to its use of specific targeting factors to eradicate cancer more efficiently and safely with fewer adverse events. Some examples of immunotherapy modalities include monoclonal antibodies (mAb) and cancer vaccines. Monoclonal antibodies have been genetically modified in order to target specific cancers such as neuroblastomas and other types of brain cancers. Cancer vaccines have also emerged in order to alter the tumor microenvironment in order to treat different types of cancers. Clinical trials are currently underway in order to test these vaccines, although anti-cancer vaccination strategies have largely been ineffective to date. Another method for cancer management is known as adoptive cell transfer.
(ACT). This type of therapy makes use of tumor-infiltrating lymphocytes (TIL) that are taken directly from the tumor itself and reinfused into the cancer patient. Other therapies include T lymphocytes that are virally transduced with a specific, chimeric antigen receptor (CAR) also known as CAR-T. This new therapy has shown promising results. The remainder of this literature review will focus heavily on CAR-T therapy.

**Generation of CAR-T cells**

The human immune system is composed of a network of two different microenvironments of cells; the innate immune system is generally composed of anti-inflammatory cells that respond quickly to infections in a non-specific pattern while the adaptive immune system is composed of cells such as Natural Killer (NK), B, and T cells that are more specific to antigens and respond slower than the innate cells. T cells are further sub-classified as CD4+ (helper) and CD8+ (killer) T cells. CD8+ T cells are heavily implicated in tumor eradication. Typically, T cells detect tumors and become activated via a two-step process involving the T cell receptor complex (TCR); this is outlined in Figure 2.
Figure 2: TCR complex and T cell activation. a) The T cell receptor (TCR) consists of an alpha and beta chain. This binds to an MHC on an APC and is therefore HLA-dependent. The signaling comes from the CD3 complex. Upon binding with MHC, the immunoreceptor tyrosine-based activation motifs (ITAMS), depicted by the rectangles in the CD3 complex, become phosphorylated and lead to T cell activation. b) This model depicts the two-step binding process required for T cell activation. The first signal is initiated by the binding of the TCR complex to the antigenic peptide (Ag), which is presented by the APC’s MHC. The second signal is antigen independent and is co-delivered by the APC (B7) and leads to activation of T cells when bound to the main co-stimulatory receptor on T cells (CD28). This second signal increases transcription of IL-2, the stability of its mRNA, expression of BCL-X (an anti-apoptotic protein), and lowers the threshold for T-cell activation. (adapted from Sadelain et al; Targeting tumors with genetically enhanced T lymphocytes)

First, the TCR must bind to the Major Histocompatibility Complex (MHC) on an antigen-presenting cell (APC) that displays a fragmented peptide antigen from the tumor. In order for it to actually become activated and carry out an effective response, the T cell must also concurrently receive a co-stimulatory signal from a recruited CD28 receptor displayed on the T cell surface after binding with the appropriate molecules on the APC. Without this co-stimulation, lymphocytes are inactivated and either remain in that anergic state or they are deleted. The major limitations of this type of recognition and activation are MHC-restriction and the need for co-stimulation in order to become
affective; tumors are also able to escape immune recognition through the downregulation of these MHC molecules rendering them undetectable by T cells.\textsuperscript{5}

In 1989, researchers began to focus their attention on overcoming the limitations of adaptive immunotherapy by looking at the MHC-restriction of T cells.\textsuperscript{24} Gross et al. was interested in the major differences and similarities between antigen recognition of TCRs and that of antibodies. While TCRs recognize antigens via fragments from APCs, antibodies are able to bind directly to the antigen itself in its native form with a relatively high affinity. The molecular structure of the two entities, however, has been shown to be relatively similar. While TCRs have an alpha and beta polypeptide chain spanning the membrane, antibodies similarly have a L (light) and H (heavy) region also composed of disulfide-linked polypeptides; each of these peptides in TCRs and antibodies has a constant (C) and variable (V) region. The binding sites of both molecules are encoded by an exon on the V-portion of the gene. Of note, previous data have concluded that the V and C regions on TCRs are not only connected to one another spatially such as in antibodies but they also have homology to immunoglobulins and thus must be structurally similar in formation. Hence, Gross et al. concluded that the TCR V region can be manipulated to express a chimeric antibody V region by switching out one for the other; this would give T cells higher affinity for the native antigen and eliminate the need for MHC-binding as well as dependence on co-stimulatory ligands. The researchers then genetically fused the intracellular C signaling domain of the TCR (CD3ζ) with the V region of an antibody domain which was composed of a single chain variable fragment (scFv) The resulting transfected, first-generation chimeric T cells exhibited a heightened
capability to react to a broad range of antigens without binding to MHC. This was a major innovation in immunology because it enabled alteration of the genetic design of TCR and thus allowed for the exploitation of T cells in order to target specific tumors, given that those tumors can be targeted by a mAb. Nevertheless, the genetic fusion of scFv with CD3ζ only provided the first signal (the same one as when T cells bind with MHC) but not the second signal required (the one from co-stimulatory domains) and thus lead to anergy, dysfunctional cytokine release, and eventually apoptosis.

Since then, several different generations of CAR-Ts have been developed (Figure 3) with the addition of co-stimulatory domains such as CD28 to enhance T cell activation in response to tumor antigen ligation.

![Figure 3: Generations 1-4 of CAR-T](adapted from Haji-Fatahaliha et al; CAR-modified T-cell therapy for cancer: an updated review)
In 2007, Brentjens et al. conducted a study using second generation CAR-Ts that targeted NALM-6-expressing tumors in mice which are the model correlates of CD19 B cell tumors in humans.26 Like B cell leukemias in humans, these mouse tumors also lack the signal for co-stimulatory ligands, minimizing the efficacy of CAR-T. The researchers found that the CAR-T were able to completely eliminate systemic tumor cells despite lack of co-stimulatory ligands as well as lack of in vivo cytokine secretion. Their findings delineated the necessity for in vivo T-cell persistence via multiple infusions in order to increase the efficacy of adoptive immunotherapy in systemic tumor eradication.26

Carpenito et al. attempted to address the limitation of inadequate in vivo persistence of previous CAR-T cells by constructing a 3rd generation of CAR-T that targeted mesothelin, a common target of carcinomas.27 CD28 and CD137 domains were fused attached to the T cells for co-stimulatory support. The results showed higher rates of T cell survival, increased cytokine production, and enhanced tumor eradication. The efficacy of this study design could have been attributed to a number of reasons. First of all, these genetically modified cells were transduced with a lentivirus (as opposed to a retrovirus as had been done in previous studies). The researchers thought that the lentiviral vectors, since they are more efficiently transduced, lead to shorter in vitro culture times and ability to use the CAR-Ts at an earlier time, which correlate to decreased telomerase activity and thus allows longer telomere sequences. Longer telomere lengths allow for a higher degree of replication. Similarly, previous studies have shown that using “younger” lymphocytes correlates with enhanced tumor eradication. Secondly, this study suggested that since CD28 increases T cell resistance and in vivo
antitumor killing and CD137 is necessary for engraftment, CAR-T efficiency would be enhanced with the use of both co-stimulatory domains as opposed to having just one or the other. This study depicted even further, the antitumor potential of CAR-T.²⁷

Eventually the most recent, fourth-generation CAR-Ts were developed that were redirected for universal cytokine-mediated killing (TRUCK); in addition to containing three co-stimulatory domains, they have the intrinsic ability to release IL-12, a crucial cytokine in recruiting inflammatory cells to the tumor site.²⁸ Chmielewski and colleagues noted that as cancer progresses, tumor cells have the ability to decrease expression of MHC as well as other antigens, thus decreasing detection by cytotoxic T and other immune cells. They combined the action of a CAR and multiple co-stimulatory domains, with immediate, local release of IL-12 that would recruit macrophages to the tumor site in order to enhance tumor cell death. This decreased the effects of systemic IL-12 toxicity by locally restricted release of IL-12 only when the T cell was activated. Also, this helped to deliver the cytokinines directly into the tumor microenvironment in a continuous manner, allowing constant cytokine release plus immune cell recruitment and activity against the tumor while decreasing the rate of CAR-T apoptosis. In addition, since innate immune cells are being recruited to the tumor site, their action of killing is not dependent on antigen, so although cancer cells are able to downregulate antigen expression on their cell surfaces, the antitumor actions of these inflammatory cells (usually macrophages) can still take place.²⁸
Clinical Application

Clinical trials eventually emerged, testing the safety and efficacy of the different generations of CAR-T. By 2005, there were only a handful of Phase I clinical trials that showed the safety of genetically-modified T cells. At that point, it was deemed safe to use high amounts of the transfused cells (>10^9). The patients that were started on CAR-T therapy went through a lymphodepleting process before T cell transfer in order to eliminate T regulatory cells and other lymphocytes that might compete for cytokines. CAR-T was also given in combination with exogenous IL-2 administration to promote persistence of the cells. Nevertheless, there were no tumor responses in these individuals with progressive disease before 2005. More clinical trials have emerged since then that differed in their targeted antigen, method of transducing the T cells, addition of IL-2 infusions, dosage, and accompanying conditioning therapy.

CAR-T in B-cell malignancies

To date, immunotherapy has proven to be relatively safe and efficacious in treating certain leukemias. One study assessed the clinical activity of CAR-T in patients with Chronic Lymphoid Leukemia (CLL). A single patient with progressive CLL (refractory to Rituximab plus Fludarabine, Rituximab plus Bendamustine, Bendamustine alone, and Alemtuzumab) was infused with second-generation autologous CAR-T specific for CD-19 B cells. These CAR-Ts were transduced with a lentiviral vector (as opposed to the traditional retroviral vector) that coupled a costimulatory receptor (CD137) with a signaling domain (CD3-zeta) in order to increase its specificity for the CD19 receptor and eliminate dependence on HLA domains. The patient was treated with
lymphodepleting chemotherapy four days prior to low-dose CAR-T infusions, which is believed to potentiate the ability for CAR-T to kill tumor cells. CAR-T were infused over three consecutive days at a total low dose of $1.5 \times 10^5$ cells.\(^4\)

Several days later, the patient developed progressively worsening systemic symptoms including chills, fevers, fatigue, nausea, and eventually tumor lysis syndrome (TLS) on post-infusion day 22 which were reversed with fluids and rasburicase.\(^4\) It should be noted that the patient did not receive supplemental cytokine administration at any point during the clinical trial. Twenty-eight days after the last infusion of CART19 cells, a bone marrow biopsy, FISH testing, and flow-cytometric analysis revealed absence of CLL cells, a negative test for deletion of TP53, and total absence of B cells, respectively. Physical exam showed no adenopathy. The same findings were evident 3 and 6 months post CAR-T infusion and the patient remained in remission at the time of the study’s publication, 10 months post-infusion.\(^4\)

A second study conducted 2 years later by the same group of researchers attempted to use CART therapy in two pediatric patients with relapsing Pre-B cell Acute Lymphocytic Leukemia (ALL) refractory to chemotherapy.\(^3\) This type of cancer is typically extremely aggressive and is accompanied by a poor prognosis. The study design was similar to the CLL clinical trial\(^4\) in that patients were treated with the same second-generation CAR019 T-cells over 3 consecutive days, with the exception of differing doses. The first patient’s disease was previously non-responsive to intensive chemotherapy consisting of clofarabine, etoposide, and cyclophosphamide. Therefore, it was not necessary for her to receive chemotherapy before CAR-T infusion. This patient
eventually developed high-grade fevers 4 days after infusions and required hospitalization to the ICU, mechanical ventilation and intensive interventions to lower her blood pressure. The effects of the cytokine release syndrome were rapidly reversed with etanercept and tocilizumab.³

The second patient had ALL refractory to umbilical cord-blood transplantation and treatment with blinatumomab.³ Her disease was unresponsive to the therapy, so her intervention consisted of chemotherapy (etoposide-cyclophosphamide) followed by a one-time dose of 10⁷ CTL019 T cells. This patient also developed a fever 6 days post infusion, most likely from Cytokine Release Syndrome (CRS) and she was hospitalized.³ Both patients suffered grade 3-4 adverse events as well as CRS that was reversed with Etanercept plus Tocilizumab.³ Both patients had complete remission immediately after therapy that was characterized by a decrease in tumors markers detected by flow cytometric assays. Most of the circulating lymphocytes in both patients were found to be CAR-T; interestingly enough, CAR-T were also found in the CNS of both patients, even though no disease was found there before infusion. While one patient’s disease did relapse, the second patient was disease free for an ongoing time of 11 months post CAR-T infusion.³

**CAR-T in solid tumors**

Adoptive cell therapy (ACT) had emerged as a very promising form of immunotherapy for solid tumors such as metastatic melanoma.³¹ This requires resection of tumor nodules in order to collect autologous TILs from the site. After TILs are collected, they are expanded *in vitro* then rein infused back into the patient. Prior to any of
this, it is important that the patients undergo lymphodepletion in order to eliminate the competition for homeostatic cytokines by T regulatory cells and other lymphocytes. While some studies exhibited a certain degree of objective response, several drawbacks exist with this therapy. The TILs were difficult to collect and did not always persist in vivo. Also, resection is required where some diseases are too extensive to resect.

CAR-T eventually emerged afterwards. The efficacy of CAR-T for solid tumors has been shown in preclinical and animal experiments. One example is in ovarian cancer. A majority of ovarian carcinomas exhibit the mucin biomolecule, more specifically MUC-16, on their cell surface. Chekmasova et al. conducted an experimental design where they transduced human T cells with chimeric antigens specific for MUC-CD (an extracellular domain located on MUC-16) in SCID-Beige mice bearing the MUC-CD+ tumors. The results exhibited cytolytic activity against ovarian carcinoma cells in vitro. Also, when these T cells were infused into the mice, this lead to either stable disease or total eradication of disease. However, while efficacy has been shown in preclinical experiments, so far only the safety of CAR-T has been established in clinical trials treating solid tumors; the outcomes and successes are marginal. To date, CAR-T has been used for a number of solid tumors including sarcomas, CEA+ liver metastases, mesothelioma, neuroblastoma, colon cancer, and ovarian cancer.

A study by Lamers and colleagues attempted to treat 12 patients with metastatic renal cell carcinoma (RCC) that expressed carboxylyhydrase IX (CAIX). Patients were divided into 3 cohorts and given 8-10 CAR-T infusions varying from 0.2 to 2.1 x 10⁹ cells. Four patients in the first 2 cohorts experienced grade 2-4 liver toxicities even with
the lowest dose of CAR-T; they had to discontinue therapy. The study did not yield any clinical responses but it did demonstrate the potency of CAR-T in metastatic RCC even in the lowest doses.\textsuperscript{35} While some of these studies indicate some degree of anti-tumor efficacy, such as the study treating neuroblastoma with CAR-T targeting CD171, it is minimal and they mostly illustrate overall safety with using CAR-T.\textsuperscript{33} CAR-T for CEA+ liver metastases is discussed in a separate section below.

Several challenges attribute to the lack of clinical response in solid tumors compared to liquid ones. First of all, only a limited number of antigens are specific to solid tumors.\textsuperscript{5} Whereas T cells can be transduced with a number of different vectors for leukemias, solid tumors only respond to unique antigens making it more difficult to screen for the proper receptors that will target the tumor while sparing healthy tissue. In order to do this effectively, researchers claim it is important that CAR-T targets neoantigens, which are short peptides created by genetic mutations of malignant cell genomes; they are not present in normal tissue.\textsuperscript{36,37} In addition, co-stimulatory molecules pose a challenge for CAR-T in that it still remains unclear which domain is superior in increasing CAR-T expansion. While it has been agreed upon that inclusion of a co-stimulatory domain does in fact influence the therapeutic response, studies are inconsistent about whether CD28 is superior to 4-1BB or vice versa.\textsuperscript{38} Recent studies suggest that CD28 accelerates expansion to the point that T cell exhaustion and thus decreased persistence occur\textsuperscript{39} and also that 4-1BB is responsible for the promotion of memory T cells while CD28 promotes naïve T cells which are less effective in tumor eradication.\textsuperscript{40} Another study indicates that cytotoxicity \textit{in vivo} and \textit{in vitro} were not
significantly any different with either domains but CD28 did produce higher amounts of cytokines.\textsuperscript{27} One other issue limiting efficacy in solid tumors is the processing of CAR-T.\textsuperscript{5} Migration of CAR-T to tumor sites is dependent on factors such as integrins, chemokines, and receptors for these molecules.\textsuperscript{41} These allow the CAR-T cells to traffic and accumulate around the target tumor cells, permitting them to carry out their anti-tumor functioning, despite the tumor microenvironment. However, altering the genetic structure of T cells may in turn decrease expression of these chemokine receptors and decrease the ability for CAR-T to find the tumor site.\textsuperscript{42}

\textit{Immunosuppression in the Liver}

The immunosuppression that takes place in the liver is a specific reason why it is difficult to target liver tumors with immunotherapy. The liver plays a major role in any individual’s immune system.\textsuperscript{43} It controls autoimmune responses to various antigens, protects against certain pathogens, and is also capable of regulating peripheral tolerance. In an attempt to assess how CD4 T cells from the liver function differently than those from other organs, Katz et al. removed bulk and CD4 T cells from mouse livers as well as from their spleen and compared them in terms of their responses to antigenic stimulation. They hypothesized that conventional liver T cell functioning is suppressed by the T cell’s native environment. Their findings showed that liver CD4 T cells were different in that they produced higher levels of immunosuppressive cytokines. Results also showed that when infused with Natural Killer Cells and \( \gamma \delta \)T cells \textit{in vitro}, conventional T cell functioning was suppressed and proliferation was halted in a dose-dependent fashion in both the liver and spleen. Other factors may have been responsible for this suppression,
however, including cytokines and other cells of the immune system including sinusoidal endothelial cells (which can induce anergy) and hepatocytes.\textsuperscript{43}

For the most part, when liver T cells are stimulated within their microenvironment, this leads to tolerance both within the liver and systemically.\textsuperscript{44} This was portrayed when the delivery of antigens or allografts to the liver failed to induce attacks by the immune system. Liver allografts, for example, have been readily accepted in numerous cases. Tolerance is thought to be promoted through various mechanisms including but not limited to evasion of the immune system, anergy, apoptosis, and the promotion of regulatory T cells. Priming of mature T cells in lymphoid tissue as well as naïve cells in the liver can also result in tolerance. The sinusoidal architecture of the liver plays a role in that it promotes priming of lymphocytes, which eventually leads to tolerance. While this may be a positive feature of the liver in terms of therapies for autoimmune diseases, it can also be negative in that certain types of immunotherapy, including the administration of CAR-T, may impede the liver’s ability to induce an immune response, allowing tumors or other diseases such as a viral hepatitis to continue growing or to last for longer periods of time.\textsuperscript{44} Nevertheless, the exact mechanisms and consequences of hepatic tolerance must be further investigated in order to create new therapies for diseases involving liver.

\textit{CAR-T in CLM}

The immunologic glycoprotein, CEA, is not only expressed by non-cancerous epithelial cells of the colonic crypts in the gastrointestinal tract, but it is also over-produced in several epithelial malignancies, especially colorectal adenocarcinoma,
making this tumor marker an attractive target for immunotherapy.\textsuperscript{45} CAR-T cells that readily recognize CEA have been developed by retroviral transduction of the TCR from the splenocytes of HLA-A2.1 transgenic mice into human PBLs after substituting a single/dual amino acid into the complementarity determining regions (CDR) of the alpha and beta domains of the murine TCRs.\textsuperscript{46,47} This would allow the CAR-T to target cancer cells expressing CEA \textit{in vitro}.

A study designed by Parkhurst et al. treated 3 patients harboring CEA+ CLM refractory to chemotherapy treatment with these autologous CAR-T targeting CEA-expressing cells in combination with IL-2 administration, post lymphodepleting chemotherapy.\textsuperscript{45} While all 3 patients had significant decreases in CEA levels (a 74-99\% drop), the levels rose back up again after several months. Two of the patients did have a decrease in their metastases (as determined by RECIST criteria), but they eventually developed progressive disease by 5-6 months post CAR-T therapy; the third was unresponsive to therapy. The fact that normal cells of the GI tract also express CEA, as previously mentioned, helps to explain why all 3 of these patients also developed varying degrees of transient colitis. Approximately a week after last CAR-T infusion, colonoscopies revealed edematous, ulcerated, denuded mucosa and biopsies demonstrated acute/chronic inflammation of the atypical epithelium. Budesonide and Mesalamine were used to treat two of the patients.\textsuperscript{45}

Katz et al. conducted a Phase I Hepatic Immunotherapy for Metastases (HITM) clinical trial where 6 patients with CEA-expressing liver metastases (secondary to primary colorectal adenocarcinoma and pancreatobiliary ampullary carcinoma) were
treated with hepatic CAR-T immunotherapy. Five out of 6 patients had over 10 liver metastases before entrance into the HITM trial; their disease was advanced, non-resectable, and refractory to several trials of chemotherapy. Upon recruitment, the patients were divided into two cohorts; the first cohort received dose-escalation x3 HAI CAR-T specifically targeting CEA+ tumors while the second cohort was treated with maximum doses (10^{10} cells) x3 of HAI CAR-T in combination with systemic IL-2 infusion. Patients were not pre-treated with lymphodepleting chemotherapy. Adverse events were limited to grades 1-3 fever, tachycardia, increased liver enzymes, diarrhea, and abdominal pain. Results showed an increase in interferon gamma (IFNγ) levels as well as a 37% decrease in CEA levels in the second cohort; there were transient, less remarkable decreases in cohort 1. Liver biopsies showed necrotic liver metastases in 3 patients. Death eventually ensued in 5 patients secondary to disease progression and the last remaining patient had stable disease even at 38 months follow-up since last CAR-T infusion; radiographic tests showed no evidence of disease progression in this patient. Overall, this study showed that HAI CAR-T targeting CEA+ tumors is safe in patients with advanced metastatic liver disease and has opened the way for further research in targeted immunotherapy.

Katz et al. are conducting another study that is currently recruiting patients. This phase Ib clinical trial, known as the HITM-SIR trial, combines the same regimen of HAI CAR-T as previously stated with a one-time intra-hepatic administration of SIR-spheres Y-90 microspheres. Selective Internal Radiation Therapy (SIRT) otherwise known as Radioembolization is a method of delivering Yttrium-90 (β-emitting isotopes) directly
into the microvasculature enclosing non-resectable liver tumors. This is done via interventional radiology-guided micro-catheter placement into the hepatic artery and delivery of these microspheres to pre-specified tumor-burdened areas of the liver. Their size (32.5 microns in diameter) allows them to lodge in distal branches (arterioles, typically) of the hepatic artery, allowing them to effectively deliver high-energy tumoricidal radiation close to targeted areas without affecting surrounding healthy liver tissue. This type of therapy is typically indicated in patients with liver metastases deemed non-resectable and is usually administered as a single dose only with adjuvant systemic chemotherapy. Adverse events include fibrosis, portal hypertension, parenchymal volume changes of the liver. By combining this modality, the tumoricidal action of CAR-T would be further enhanced.
METHODS

Study design

The study design will be based on a Katz et al.’s Phase I HITM and currently ongoing HITM-SIR clinical trials.\(^7\)\(^8\) Further information regarding the HITM-SIR trial can be found at clinicaltrials.gov. This design will be a Phase II non-randomized, single-armed, interventional clinical trial using CAR-T therapy targeting CEA+ hepatic, unresectable metastases in combination with administration of a one time dose of SIR-Spheres Y-90 resin microspheres. 63 patients will be recruited to receive 3 intrahepatic infusions of the maximum dose \( (10^{10}) \) CAR-T every two weeks. The microspheres will be administered two weeks following the last CAR-T dose resulting in a 6-week treatment course; IL-2 will be given continuously throughout the 6 weeks. Imaging with MRI/PET will be done one month before, at 6 weeks, and three months after CAR-T therapy. After the three month mark, MRI will be performed every month for a year then every 3 months for a year and PET every 6 months for those two years in order to assess PFS. Findings from this trial will be compared with the randomized, phase III SIRFLOX clinical trial.\(^5\)\(^3\) This trial consisted of two treatment arms for patients with metastatic CRC; the first was given FOLFOX while the second was given FOLF\( OX \) in combination with SIRT; median PFS was found to be 10.2 and 10.7 months respectively.\(^5\)\(^3\)

Study population and sampling

Historical control data from the SIRFLOX study\(^5\)\(^3\) will be used to compare results and to determine sample size. The second cohort group of combination FOLFOX and
SIRT had 10.7 months median PFS in a total of 276 participants. For this current trial, a sample size of 63 participants with CEA+ liver metastases from a primary colorectal adenocarcinoma will need to be recruited (alpha of 0.05, power of 80%, and standard deviation of 2 months) in order to detect a 10% increase in PFS months making expected median survival of this study 11.7 or close to 12 months.

**Recruitment**

Health care providers treating patients with extensive disease will be able to offer this therapy for anyone interested that also meets the inclusion criteria. Inclusion and exclusion criteria for all participants can be found in Appendix I. Recruitment will take place after contact with various hospitals/medical centers and health care providers, inquiring about patients that may be eligible. Presentations will also be given at various conferences/meetings in order to make providers aware of this trial and as a chance to recruit patients. This study will also be uploaded to clinicaltrials.gov. If individuals do meet appropriate criteria, they will be offered the choice of undergoing treatment. They will be contacted via telephone or seen in the clinic by their oncologists who will be well informed about this clinical trial. Benefits and drawbacks will be discussed extensively. Patients will be able to ask questions and will be told in full detail the implications and timeframe of this clinical trial. Written and signed consent for interested participants will be gathered before induction into the study.
Treatment

Upon acquisition of consent, eligible subjects meeting the inclusion criteria will receive a PET and liver MRI at least a month prior to start of treatment; number and diameters of liver metastases will be recorded. In addition, CEA levels will also be measured (levels should be >10ng/mL) at the same time. Prior to therapy initiation, patients will undergo angiography in order to assess hepatic artery anatomy to ensure that it is adequate for arterial infusions. Study subjects then will receive three doses of CAR-T cells ($10^{10}$ cells) with a continuous, systemic IL-2 administration (50,000 U/kg/d) given over the course of 6 weeks via an ambulatory infusion pump. CAR-T will be administered in two-week increments on days 1, 14, and 28 via percutaneous HAI directly into the liver’s circulation. This will be done with 100mL at each administration at an injection rate of <2mL/second. At the third and last administration of CAR-T, patients will receive a follow-up PET, liver MRI, normal liver core needle biopsy as well as tumor core needle biopsy. These tissue samples will be stained with H&E (hematoxylin and eosin) and anti-CEA antibody and observed under the microscope for evidence of necrosis and fibrosis. Flow cytometry of biopsy tissues will be used to detect the presence of CAR-T. These findings will be compared to the findings obtained before initiation of therapy. Two weeks following the last CAR-T dose, patients will receive a one-time administration of localized SIR-spheres via interventional radiology-guided micro-catheter placement into the hepatic artery. The procedure will take roughly an hour and after getting scanned with CT, to confirm proper placement of the microspheres,
patients will be able to return home. Three months later, patient’s serum will be screened for presence of anti-CAR antibodies, IFNγ levels, and patients will again receive a follow up liver MRI and PET which will be graded by two radiologists in blinded fashion. Participants will receive a follow up MRI every month thereafter for the next 12 months, given that disease does not progress. Once disease is deemed progressive, imaging will cease. If disease is stable, frequency of MRI will drop down to every 3 months for 12 months after that. PET scans will be performed every 6 months during those 24 months.

Patients will be monitored for CRS and assessed for other adverse events using the National Cancer Institute’s CTCAE v4.055 as well as the Interim Monitoring Protocol. Adverse events are graded on a scale of 1-5 delineating mild, moderate, severe, life-threatening/disabling, and death-related adverse events respectively. Should patients develop severe or life-threatening CRS or other adverse events, treatment with CAR-T will be stopped and patients will be treated with the proper protocol for such events.

*CAR-T production in the lab*

This study will utilize leukapheresis at the various medical centers where subjects are recruited in order to collect T cells from patients; this will last somewhere from 2-3 hours. After isolating the peripheral blood mononuclear cells (PBMC), the cells will be activated with AIM V media, human AB serum, anti-CD3 mAb, and IL-2 for the next 48-72 hours. The T cells will then be retrovirally transduced three times in the lab with a tandem molecule formed from the fusion of the hMN14 sFv-CD8 hinge region of the viral backbone with a hybrid CD28/CD3d fragment; this is to be done in a facility capable of processing and manufacturing these CAR-T as well as storing and distributing
them back into patients. This process leads to the production of second-generation CAR-T targeting CEA and it will take roughly 24 hours. The CAR-T will then be washed, incubated, and expanded in growth media over a period of 12-17 days before they are examined via flow cytometry with specific antibodies for CD3, CD4, CD8. After clinical doses of CAR-T are prepared, they will be frozen, stored, and thawed when ready to be infused.

**Study variables and measures**

The primary endpoint assessed will be PFS in months. After imaging is performed via PET and liver MRI, responses will be graded by two radiologists blinded to the trial (a third blinded radiologist will be recruited should the original two disagree on a particular finding) using mRECIST. This criteria focuses on target lesions, which are characterized by the largest metastases in diameter that can be found; changes in these target lesions are quantified by taking the sum of the diameters of viable tissue and averaging them. The average or percent change will depict the tumor response at that time; all follow up measurements will be compared to those pre- and immediately post-therapy. Definitions of tumor responses are summed up in Table 1. The same criteria exist for Hepatocellular Carcinoma (HCC) as for liver metastases. Progression is defined as a ≥ 20% increase in the average of the summed diameters of viable tissue (target lesions). The time to progression will be recorded for all study subjects and compared to historical data. Secondary outcomes include tumor response rate, extra and intrahepatic tumor recurrence rate, adverse events, and overall survival (OS).
Table 1: Tumor response definitions for HCC according to mRECIST (adapted from Fournier et al, Imaging criteria for assessing tumor response: RECIST, mRECIST, Cheson)\textsuperscript{36}

<table>
<thead>
<tr>
<th>Definition</th>
<th>Target lesions</th>
<th>Non-target lesions</th>
<th>New lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC longest diameter $\geq 10$ mm nodular (clear boundaries, non-infiltrating) enhancement on arterial phase on CT or MRI For other sites: id. RECIST</td>
<td>HCC: lesion too small ($&lt; 10$ mm), infiltrating or atypical enhancement (non-arterial) For other sites: id. RECIST</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Complete response (CR) Disappearance of any intratumoral arterial enhancement during in target lesions | Id. RECIST | No (no new lesion) |

Partial response (PR) $\geq 30\%$ of the sum of the diameters of viable portions (enhancement on arterial phase) of target lesions taking as reference the baseline sum | Id. RECIST | No (no new lesion) |

Stable disease (SD) Neither response nor progression | Id. RECIST | No (no new lesion) |

Progressive disease (PD) $\geq 20\%$ of the sum of the diameters of viable (enhancing) portions of target lesions taking as reference the smallest sum of the diameters of viable portions of target lesions recorded since the start of treatment (nadir) | Id. RECIST | Yes (appearance of new lesion(s) for which the diagnosis of HCC is unequivocal) |

HCC: hepatocellular carcinoma.

Data collection

Data will be collected and recorded at various times throughout the study. Before treatment is initiated, CEA levels and number and diameter of metastases will be recorded. After treatment, measurement of remaining and/or new tumors will be recorded using the mRECIST as well as anti-CAR antibodies, CEA, and IFN$\gamma$ levels. Data on adverse events will also be collected throughout the study using CTCAE v4.0.

Data analysis

The main purpose of this study is to further analyze the safety and efficacy of using CAR-T therapy in combination with radiation therapy in a larger study population. Although limited data is available, PFS and other results will be compared with the median PFS of the SIRFLOX study (FOLFOX and SIRT arm as control) for liver
metastases. Adverse events will be graded. Response rates will be compared with a test of proportions. Time to event endpoints will be compared with the log rank test. A trial statistician will carry out data analysis.

**Timeline and Resources**

In the summer of 2016, the study proposal will be submitted to the IRB. Upon approval, several weeks of design planning will take place. Once patients begin to be recruited, the preparation of CAR-T will take roughly 3 weeks and will be performed in the laboratory. The duration of this study from start (first PET and MRI) to treatment to last follow-up MRI will be around 2 years and 5 months. However, it should be noted that it may not be feasible or likely for all the patients to start treatment at the same exact time due to lag in recruitment; therefore it can be expected that 3-5 years will be allotted for the recruitment, treatment, follow-up, and completion of this study in order to achieve the appropriate sample size.

Resource requirements for study completion include access to: personnel to perform leukopheresis; a Good Manufacturing Practice (GMP) Center with the proper equipment/supplies (tissue culture flasks, AIM V media, human AB serum, anti-CD3 mAb, IL-2, retronectin, protamine sulfate, fluorescently labeled antibodies, endotoxin assays, machine for flow cytometry, CAR detecting reagents, system to harvest cells, freezing media) and protocols to carry out manufacturing and preparation of CAR-T; a medical center to carry out angiography, imaging modalities such as PET/MRI/CT, and liver biopsies with a pathology center to stain tissues and look at them under a
microscope; and lastly, an interventional radiology department for administration of SIR-Spheres. The study will require a trial manager that will be heavily involved in the trial design phase and will be responsible for raising awareness of the clinical trial by giving presentations and conducting discussions at various conferences as well as managing trial budgets. The rest of the management team will consist of a Principal Investigator, statistician, trial programmer, data manager, data clerks, and administrative staff; these positions are responsible for various tasks including project management, sampling, data collection, data entry, and analysis. Other responsibilities include: CAR-T production in the lab, radiologists to image and biopsy patients as well as administer radiation therapy, a pathologist to view tissue specimens, and a radiologist familiar with mRECIST to grade tumor changes. Regular meetings will be arranged with all team members in order to discuss trial initiation, planning, execution, monitoring, and analysis as well as to bring up updates or any issues encountered to the entire team.

**Institutional Review Board**

Before initiating any portion of this study, permission must be obtained from the IRB before commencing study to approve non-exempt research before beginning trials on human subjects. Submission forms and proper documentation will be submitted to the Boston University Medical Center Institutional Review Board (BUMC IRB). The BUMC IRB will then pre-review the protocol and determine whether it is appropriate to be reviewed by the Western IRB, which requires submission of other forms. After approval from the WIRB, the trial can then be initiated.
CONCLUSION

Discussion

Recent advances have been made in immunology regarding the use of CAR-T therapy in liver metastases from colorectal adenocarcinoma. The safety of this therapy has thus far, been established. However, studies testing its efficacy are still underway. This is the first phase II clinical trial using regionally infused CAR-T in combination with SIR-spheres and continuous IL-2 in patients with unresectable, progressive CLM secondary to CRC. Hopefully it will demonstrate the efficacy of this treatment regimen in either keeping disease stable or lead to tumor regression in these patients, thereby prolonging PFS. While general life expectancy among this population is roughly 6-12 months before recruitment, results may exhibit increased survival, which may thus in turn, increase quality of life during this time.

The process of retrieving T cells from patients is rather safe and can be done in a few hours without any major side effects. The processing and transducing of the T cells also can be completed in a reasonably short amount of time in the lab; the manufacturing method used in this study only takes roughly 3 weeks for T cells to be collected, virally transduced, and grown to appropriate, therapeutic numbers before being easily infused back into the patient. Also, by injecting the CAR-T directly into the hepatic circulation instead of administering them systemically, this would allow a more concentrated response against the liver tumor while diminishing systemic adverse events. By adding SIR-Spheres to the treatment regimen, this will amplify tumoricidal effects while decreasing radiotherapy side effects to healthy tissue. Unfortunately the lab equipment
and supplies/treatment needed for this study are expensive, therefore, budgeting will be a crucial factor that can not be overlooked.

Following up every month with MRI will allow radiologists to pick up the first signs of tumor progression as CLM in the liver progresses rather quickly. Since the study aims to detect a 10% increase in PFS (in months) compared to the SIRFLOX study, this equates to one more month without disease progression; thus checking for this with MRI and the mRECIST protocol would allow for that. Also, MRI is relatively safe and there is no evidence that it uses ionizing radiation. That being said, participants might consider monthly MRIs a hassle. In addition, while it is expected that this treatment regimen will increase PFS, it may be that some participants do not react at all and that their metastases will neither regress nor become stable by the time treatment is finished.

Another challenge that might be expected to arise is in acquiring the appropriate sample size for this study to be carried out; this may be due to decreased awareness of the trial or hesitancy on the part of participants to enter the trial. Also, if the appropriate sample size is reached, it may in fact take longer than 5 years to recruit participants.

Some limitations exist that pertain to solid tumors specifically. While the efficacy of CAR-T in solid tumors has been demonstrated in animal models and in the lab, clinical trials have not been able to emulate these findings to a significant degree. Reasons for this have to do with the proliferation, persistence, and trafficking of CAR-T and these factors are heavily affected by the tumor microenvironment and immunosuppression in the liver. While the barriers of delivery are being overcome in this study with intrahepatic
infusions directly to the liver circulation, persistence of CAR-T has been inconsistent; persistence will be measured with fluorescently-labeled antibodies against CAR-T.

Cytokine signaling has been another issue; T cells need cytokine signaling to boost their activity \textit{in vivo} and to persist. Thus it is reasonable and necessary to give IL-2 along with CAR-T. However, as it has been evident from the current literature, cytokines can cause a number of adverse events. In addition, once the treatment ends and cytokines are no longer given, T cells cease to persist at high, efficacious levels. Future trials may be designed to avoid this by using 4th generation TRUCK T cells with intrinsic cytokine signaling.

\textbf{Summary}

CLM is a detrimental manifestation of CRC. Once disease progresses to the liver, death will almost always ensue, despite multiple rounds of systemic chemotherapy and radiation. While resection remains the only curative modality for CLM from CRC, it is not always feasible. Immunotherapy has been showing promising results in the treatment of CLM. CAR-T has already demonstrated complete remission in several B cell malignancies and it has also lead to stable disease in at least one patient with CLM. While challenges exist, there are also numerous benefits to using CAR-T therapy for cancer treatment including independence from HLA/MHC binding, easy transduction of T cells with viral vectors for genetic modification, and ability to use a number of various co-stimulator molecules to provide the second signal T cells require for activation. All of these characteristics allow greater affinity of CAR-T for tumors, greater specificity for
certain tumors by regulating co-stimulatory molecules, and greater prevention for escape of tumor cells from immuno-regulation by down regulating surface antigens. All of these characteristics are of great importance in solid tumors.

Clinical and/or public health significance

The cure for cancer is an incredibly important societal goal and even with the medical advances that exist today, some cancers are much more difficult to treat than others. The aims of this study have been to test the efficacy of using CAR-T immunotherapy in combination with localized radiation therapy in the hopes that this treatment modality would essentially prolong progression free survival in patients with CLM. This would hopefully not only increase quality of life for these patients but would give researchers an insight into genetic modification of the immune system to aid future studies. Hopefully, further investigation and further clinical trials in immunotherapy will soon allow complete remission in not only liquid but also solid tumors as well.
### APPENDIX

#### I. Inclusion and Exclusion Criteria for Current Trial

<table>
<thead>
<tr>
<th>INCLUSION CRITERIA</th>
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<tbody>
<tr>
<td>Patient with histologically confirmed diagnosis of CEA+ adenocarcinoma and liver metastases. Patient must have either histologic confirmation of the liver metastases or histologic documentation of the primary tumor and definitive radiologic evidence of liver involvement. Measurable disease is required with lesions of &gt; 1.0 cm by CT. Soluble CEA is not acceptable as the sole measure of disease. Limited extrahepatic disease is acceptable if confined to the lungs or peritoneal cavity.</td>
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<tr>
<td>Tumor must be CEA-expressing as demonstrated by elevated serum CEA levels (≥10ng/ml) or immunohistochemistry on a biopsy specimen. Archived tissue is acceptable for determination of CEA expression.</td>
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<td>Patient must be at least 18 years of age.</td>
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<td>Patient able to understand and sign informed consent.</td>
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<td>Patient with a life expectancy of greater than four months.</td>
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<tr>
<td>Patient failed at least one line of standard systemic chemotherapy and has unresectable disease.</td>
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<tr>
<td>Patient with adequate organ function</td>
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<tr>
<td>Acceptable hepatic vascular anatomy as determined by CT, MR, or conventional angiography. A nuclear medicine study will be performed to document the absence of a significant hepatic-pulmonary shunt (&lt;20%).</td>
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<tr>
<th>EXCLUSION CRITERIA</th>
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<tbody>
<tr>
<td>Pregnant patients will be excluded from the study. Males who are actively seeking to have children will be made aware of the unknown risks of this study protocol on human sperm and the need to practice birth control.</td>
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<tr>
<td>Patients with serious or unstable renal, hepatic, pulmonary, cardiovascular, endocrine, rheumatologic, or allergic disease based on history, physical exam and laboratory tests will be excluded.</td>
</tr>
<tr>
<td>Patients with active clinical disease caused by CMV, hepatitis B or C, HIV or tuberculosis will be excluded from the study.</td>
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<tr>
<td>Patients who have had cytotoxic and/or radiation therapy within 4 weeks prior to entry into the trial or 4 weeks prior to infusion will be excluded. Patients with other concurrent malignancies will be excluded.</td>
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<tr>
<td>Patients requiring systemic steroids will be excluded.</td>
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<tr>
<td>Patients with unsuitable hepatic vascular anatomy will be excluded from the study.</td>
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<tr>
<td>Conditions</td>
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<tr>
<td>Patients with extrahepatic metastatic disease beyond the lungs or abdominal/retroperitoneal lymph nodes.</td>
</tr>
<tr>
<td>Patients with &gt;50% liver replacement at time of treatment will be excluded.</td>
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<tr>
<td>Previous external beam radiotherapy to the liver.</td>
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<tr>
<td>Portal vein thrombosis.</td>
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</tbody>
</table>
# LIST OF JOURNAL ABBREVIATIONS

<table>
<thead>
<tr>
<th>Journal Abbreviation</th>
<th>Full Journal Name</th>
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<tbody>
<tr>
<td>Artif Cells Nanomed Biotechnol</td>
<td>Artificial Cells, Nanomedicine, and Biotechnology</td>
</tr>
<tr>
<td>BJC</td>
<td>British Journal of Cancer</td>
</tr>
<tr>
<td>CAN</td>
<td>Cancer Research</td>
</tr>
<tr>
<td>Cancer Immunol Immunother</td>
<td>Cancer Immunology, Immunotherapy</td>
</tr>
<tr>
<td>CCR</td>
<td>Clinical Cancer Research</td>
</tr>
<tr>
<td>COI</td>
<td>Current Opinion in Immunology</td>
</tr>
<tr>
<td>Dig Dis Sci</td>
<td>Digestive Diseases and Science</td>
</tr>
<tr>
<td>DII</td>
<td>Diagnostic and Interventional Imaging Journal</td>
</tr>
<tr>
<td>FEBS Lett</td>
<td>The Federation of European Biochemical Societies Letters</td>
</tr>
<tr>
<td>FIMMU</td>
<td>Frontiers in Immunology</td>
</tr>
<tr>
<td>HEP</td>
<td>Journal of Hepatology</td>
</tr>
<tr>
<td>JAUT</td>
<td>Journal of Autoimmunity</td>
</tr>
<tr>
<td>JCI</td>
<td>The Journal of Clinical Investigation</td>
</tr>
<tr>
<td>JCO</td>
<td>Journal of Clinical Oncology</td>
</tr>
<tr>
<td>J Immunol</td>
<td>The Journal of Immunology</td>
</tr>
<tr>
<td>J Immunol Res</td>
<td>Journal of Immunology Research</td>
</tr>
<tr>
<td>JLB</td>
<td>Journal of Leukocyte Biology</td>
</tr>
<tr>
<td>JVIR</td>
<td>Journal of Vascular and Interventional Radiology</td>
</tr>
<tr>
<td>Lancet Oncol</td>
<td>The Lancet Oncology</td>
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<tr>
<td>MT</td>
<td>Molecular Therapy</td>
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<tr>
<td>Abbreviation</td>
<td>Journal Name</td>
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<tr>
<td>NM</td>
<td>Nature Medicine</td>
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<tr>
<td>NRC</td>
<td>Nature Reviews Cancer</td>
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<tr>
<td>NEJM</td>
<td>New England Journal of Medicine</td>
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<tr>
<td>ONCI</td>
<td>OncoImmunology</td>
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<tr>
<td>PNAS</td>
<td>Proceedings of the National Academy of Sciences</td>
</tr>
<tr>
<td>Sci Transl Med</td>
<td>Science Translational Medicine</td>
</tr>
</tbody>
</table>
REFERENCES


CURRICULUM VITAE

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EDUCATION

2014-2016 Boston University School of Medicine, Boston, MA
Master of Science, Physician Assistant Program
Degree to be conferred September 2016
Thesis: CAR-T Cell Therapy For Liver Metastases

2008-2012 Smith College, Northampton, MA
B. A., Biology

EXPERIENCE

2012-2013 EMT-Basic
Hilltown Community Ambulance Association
Huntington, MA
Responsible for responding to calls and providing emergency medical care to the critically ill/injured in high-stress emergency situations and transporting patients to the appropriate medical facility.

2009-2013 Personal Care Attendant
International Health Solutions
Springfield, MA
Responsible for assisting clients with activities of daily living, meal preparation and grocery shopping, personal hygiene, transportation to and from appointments, and assisting with ROM and strengthening exercises to ensure proper functioning.

CERTIFICATION AND LICENSURE

2016-present Physician Assistant License, State of Massachusetts pending
2016-present Certification by the National Commission on Certification of Physician Assistants pending
2014-present ACLS Certification
2012-2014 EMT-B Certification

PROFESSIONAL ACTIVITIES

2014-present Group member
American Academy of Physician Assistants

2014-present Group member, singer
Doctor’s Notes Acapella Group, BUSM

2014-2015 Group Member
Christian Medical and Dental Associations, CMDA

PRESENTATIONS

Lashtur, N., Grand Rounds Presentation: Dermatology Roger Williams, Boston University Physician Assistant Program, Boston, Massachusetts, August 2015

Lashtur, N., Grand Rounds Presentation: Mediastinal Masses, Boston University Physician Assistant Program, Boston, Massachusetts, November 2015