Review Article

Combination of natural killer cell-based immunotherapy and irreversible electroporation for the treatment of hepatocellular carcinoma

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Abstract: Hepatocellular carcinoma (HCC) is among the most lethal cancer types despite great advancement in overall survival of the patients over the last decades. Surgical resection or partial hepatectomy has been approved as the curative treatment for early-stage HCC patients however only up to 30% of them are eligible for the procedures. Natural killer (NK) cells are cytotoxic lymphocytes recognized for killing virally infected cells and improving immune functions for defending the body against malignant cells. Although autologous NK cells failed to demonstrate significant clinical benefit, transfer of allogeneic adoptive NK cells arises as a promising approach for the treatment of solid tumors. The immunosuppressive tumor microenvironment and inadequate homing efficiency of NK cells to tumors can inhibit adoptive transfer immunotherapy (ATI) efficacy. However, potential of the NK cells is challenged by the transfection efficiency. The local ablation techniques that employ thermal or chemical energy have been investigated for the destruction of solid tumors for three decades and demonstrated promising benefits for individuals not eligible for surgical resection or partial hepatectomy. Irreversible electroporation (IRE) is one of the most recent minimally invasive ablation methods that destruct the cell within the targeted region through non-thermal energy. IRE destroys the tumor cell membrane by delivering high-frequency electrical energy in short pulses and overcomes tumor immunosuppression. The previous studies demonstrated that IRE can induce immune changes which can facilitate activation of specific immune responses and improve transfection efficiency. In this review paper, we have discussed the mechanism of NK cell immunotherapy and IRE ablation methods for the treatment of HCC patients and the combinatorial benefits of NK cell immunotherapy and IRE ablation.

Keywords: Combination therapy; hepatocellular carcinoma; irreversible electroporation (IRE); natural killer cells (NK cells)

Submitted Feb 03, 2021. Accepted for publication Apr 05, 2021.
doi: 10.21037/atm-21-539
View this article at: https://dx.doi.org/10.21037/atm-21-539

Introduction

Hepatocellular carcinoma (HCC), most common type of liver cancer, makes up to 85% of liver cancer diagnoses (1). As mainly occurring due to liver cirrhosis caused by hepatitis and alcoholic cirrhosis, HCC is ranked as the fourth leading cause of cancer-related deaths worldwide (2). Despite potential prevention of the risk factors that cause HCC, HCC surveillance has limited availability and restricts the implementation of probable curative treatment options for HCC patients (2). Surgical resection and partial hepatectomy remain effective curative treatments for early-stage HCC patients, according to the Barcelona-Clinic Liver Cancer (BCLC) disease classification (3). However, only up to 30% of the patients are surgical candidates due to multiple lesions related to chronic liver disease (4). Systemic chemotherapy increases the median survival time of the patients with advanced HCC by about three months (5,6).

Natural killer (NK) cells are cytotoxic innate immune cells that are specialized in defense against tumors and they constitute the first line of defense against invading neoplastic cells. Early studies utilizing autologous NK cell-based adoptive transfer immunotherapy (ATI) have failed to demonstrate significant clinical benefit due to the circulation of the NK cells in the bloodstream instead of tumor structures (7). However, allogeneic adoptive transfer of NK cells collected from healthy individuals to cancer patients is assumed to be a promising approach for the treatment of solid tumors including unresectable liver tumors (7,8). Yet, critical barriers, e.g., tumor immunosuppression must be overcome to achieve prominent therapeutic outcomes.

Hepatic tumor ablation, including radiofrequency ablation (RFA), microwave ablation (MWA), or cryoablation, has shown great promise for complete remission in patients with HCC smaller than 3 cm in diameter (9). However, the efficacy of these methods is challenged by location, size, and the number of tumors due to the "heat-sink" effect in which blood flow causes a cooling effect reducing the volume of ablated tumor region located near major blood vessels (10). Irreversible electroporation (IRE), a non-thermal tissue ablation technique, instigates cell death within the tumor structure and preserves the extracellular matrix, and induces minimal inflammation (11,12). Unlike other ablation techniques, IRE induces immunogenic cell death with the use of high voltage low-energy direct current pulses delivered to the treatment region with great precision while preserving vital structures such as the extracellular matrix and blood vessels within the treatment region (13,14), which is ideal for stimulating local inflammation and promoting NK cell infiltration into tumors. With the advent of immunotherapy for liver tumors, there is an increasing clinical interest in understanding the potential combination of NK cell treatment with the IRE ablation method to maximize therapeutic responses. In this review, we summarize the current investigations of NK cell-based immunotherapy and IRE ablation method as well as combination therapy (NK plus IRE) for the treatment of HCC patients.

We present the study in accordance with the Narrative Review reporting checklist (available at https://dx.doi.org/10.21037/atm-21-539).

Methods

For this study, we searched the PubMed and Web of Science databases for articles published in English during the last 20 years until August 1, 2020. The selected relevant articles were reviewed and reported in this manuscript.

NK cell-based immunotherapy

NK cells, an essential part of the innate immunity, are expressed as a member of lymphocytes that fight against tumor cells without prior sensitization (15) and are frequently located in the peritoneal cavity, bone marrow, liver, lung, lymph nodes, peripheral blood, spleen, and thymus (16). NK cells account for 20–30% of the hepatic lymphocytes in healthy human liver tissue as well as 10% of the lymphocytes in lungs (17). NK cells induce apoptosis of tumor cells through various functional mechanisms such as producing cytotoxic granules loaded with granzyme and perforin, death receptor-mediated apoptosis, secreting cytoplasmic granules, and antibody-dependent cellular cytotoxicity (18). Moreover, NK cells are suitable immunotherapeutic targets for cancer treatment, e.g., adoptive cell transfer and antibody-based strategies (19). Therefore, researchers have focused on the administration of NK cells for cancer immunotherapy. The activation of NK cells is administered by inhibitory and activating receptor signals in which NK cell activation leads to the release of granules by causing cytotoxicity with the downregulation or absence of self major histocompatibility complex (MHC) class I molecules in encountered cells (*Figure 1*) (20,21).

Several studies have reported that the malfunction of

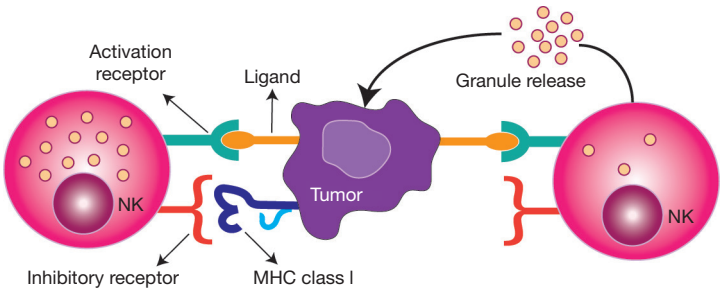

Figure 1 The activation of natural killer cells is controlled by the signals from inhibitory and activation receptors. NK cell activation is restricted as self MHC class I molecules are recognized by inhibitory NK cell receptors. In the case of downregulation or absence of self MHC class I molecules in encountered cells, NK cell activation leads to the release of granules by causing cytotoxicity. NK, natural killer; MHC, major histocompatibility complex.

NK cells was associated with the progression and metastasis of different types of cancers in both animal models and clinical studies (22-24). Particularly, increasing functional impairment and reduced proliferation of NK cells during hepatocarcinogenesis has been reported in a review article by Sun *et al.* (25). Meanwhile, Cai *et al.* observed a significant reduction of intrahepatic NK cells and a dramatic level of reduction in peripheral NK cells in HCC patients compared to healthy subjects (26). The ratio of NK cell reduction became more noticeable in the later stages of the HCC disease indicating the migration of NK cells to tumor regions (27,28). In addition, frequency and cytolytic activity of NK cells have been damaged during liver cancer (25). Several approaches have been adopted to improve the efficacy of NK cell-based immunotherapy by overcoming the lack of functioning or reduced level of NK cell presence, e.g., increasing cytotoxicity of NK cells using cytokine treatment, modulating the cytotoxic function of NK cells using antibodies, boosting NK cell-activating receptors, adoptive transfer of NK cells.

The first FDA-approved cytokine, interleukin 12, improves the cytotoxicity of the NK cells while it also improves the regulatory T (Treg) cells, which can diminish the efficacy of the NK cells (29). The study conducted by Barajas *et al.* demonstrated the possibility of treating HCC with the injection of adenovirus expressing IL-12 activating NK cells and inhibiting angiogenesis (30). On the other hand, Levin *et al.* suggested a strategy for the modification of IL-2 to prevent activation of Treg cells while maintaining an increase of cytotoxicity of NK and $CD8^+$ T cells (31). An *in vivo* study showed successful results in improving cytotoxic T cell expansion and therefore antitumor responses. Moreover, *in vitro* experiments, showed enhanced IL-2 potency, and regulated cell specificity was observed. Pillet *et al.* demonstrated the potential of IL-15 for improving NK cell activity and increasing the cytotoxicity of NK cells and $CD8^+$ T cells without simulating Treg cells (32). Furthermore, several studies investigated the strategies utilizing cytokine gene therapies in animal models for the proliferation and activation of NK cells (33-40). Tatsumi *et al.* demonstrated that intrahepatic injection of alpha-galactosylceramide-pulsed dendritic cells into the liver efficiently activated NK cells while inducing complete tumor rejection and increasing long-term survival benefits in a murine CMS4 tumor model (33). Leboeuf *et al.* have shown that human allogeneic suicide gene-modified killer cells exhibit cytotoxicity towards HCC mostly controlled by NK and NK T cells (34). Lo *et al.* highlighted the potential of IL-12 cytokines for the regulation of hepatic T cells, NK cells, and NK T cells in addition to reducing hepatic metastasis and improving survival with adeno-associated virus serotype 8/IL-12 treatment (35). The study of Gonzalez-Carmona *et al.* demonstrated that transduction of tumor-associated antigen-pulsed dendritic cells with a CD40L-encoding adenovirus provided significantly improved tumor infiltration with $CD4^+$, $CD8^+$ T, and NK cells (36). Abushahba *et al.* demonstrated that the antitumor activity of type III interferon (IFN-λ) was similar or better compared to type I interferon (IFN-α) without a direct effect on NK cells (37). Moreover, Ebert *et al.* showed a strong IFN-α response as well as antiviral activity in liver cells introduced by 3p double-stranded RNA (3p-RNA) activated retinoic acid-inducible protein I (38). Guo *et al.* targeted *Pim-3* gene by constructing a dual-function small hairpin RNA (shRNA) vector containing an shRNA for proliferation and inhibition of apoptosis (39).

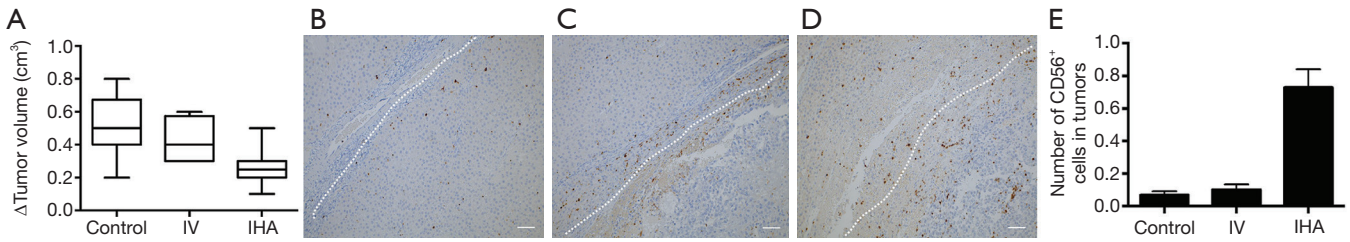

Figure 2 Natural killer (NK)-based adoptive transfer immunotherapy (ATI) outcome in a rat model of hepatocellular carcinoma (HCC). (A) The tumor volume changes after 8 days of NK cell infusion. (B,C,D) Representative CD56-stained histology slices corresponding to subjects from the control group with transcatheter intra-hepatic arterial (IHA) saline (B), intravenous (IV) NK cell (C), and transcatheter IHA NK cell infusion after 8 days of infusion (D). (E) The IHA NK cell infusion group had a significantly ($P<0.0001$) improved number of $CD56^+$ NKs compared to the control group. (B,C,D) Scale bars =100 µm. [Adapted from Su *et al.* (46), Copyright 2018 by John Wiley & Sons Ltd.]

Besides, the study of Han *et al.* demonstrated that 5'-end triphosphate hepatitis B virus X gene RNAs prevented replication of hepatitis B virus by strong expression of IFN-α and proinflammatory cytokines as well as activation of the retinoic acid-inducible gene I (40). These studies supported the potential benefits of using NK cells as a treatment approach and suggested further investigation before performing clinical trials.

The transfer of the autologous or allogeneic peripheral blood NK cells is another approach that has gained researchers' attention on cancer immunotherapy (18,41). The efficacy of transplanted NK cells is associated with killing specificity, *in vivo* activity, and persistence. Several studies emphasized that autologous NK cells could not demonstrate clinical benefits in the long-term since their anti-tumor activity is limited by the inhibitory signal transmitted by self MHC molecules (42,43). Parkhurst *et al.* used adoptively transferred *in vitro* activated autologous NK cells for the treatment of patients with melanoma and renal cell carcinoma following the lymphodepleting chemotherapy regimen (42). Despite no significant clinical benefit adoptively transferred NK cells were in peripheral circulation but they could not kill tumor cells *in vitro* without IL-2 reactivation. Sakamoto *et al.* treated locally advanced and/or metastatic digestive cancer patients using NK cells expanded *ex-vivo* with the simulation of peripheral blood mononuclear cells with OK432, IL-2, and modified FN-CH296 induced T cells (43). In addition to good-toleration of the treatment without severe adverse effects, they observed significantly increased cytotoxicity in the peripheral blood until four weeks after treatment which suggested the potential for evaluation of the approach in further clinical trials. Allogeneic NK cell infusion demonstrated an improved clinical outcome for the treatment of late-stage cancer patients including HCC (44,45) in which the procedure for NK cell isolation, expansion, and purity was critical for successful results (7). Moreover, a preclinical study performed by Su *et al.* demonstrated that further improvement can be observed with a transcatheter intra-hepatic infusion of NK cells for the treatment of HCC (46). The results emphasized the significantly increased number of $CD56^+$ NK cells in the treatment group compared to the control group in addition to reduced tumor volume growth in treated subjects (*Figure 2*).

The potential benefits of chemotherapy for augmenting NK cell efficacy have been investigated by several groups (5,47-49). Sorafenib, an FDA-approved multi-kinase inhibitor, is a standard treatment providing survival benefits for advanced HCC patients (5). As targeting vascular endothelial growth factor receptor and platelet-derived growth factor receptor, Sorafenib suppresses tumor cell proliferation and angiogenesis in addition to an increased rate of apoptosis in solid tumor models (5). In HCC, Sorafenib inhibits serine-threonine kinases Raf-1 and vascular endothelial growth factor pathways that administer cellular signaling for the molecular pathogenesis of HCC (50-53). Sprinzl *et al.* demonstrated that Sorafenib induces NK cell antitumor response through the proinflammatory activity of tumor-associated macrophages (47). In addition, Kamiya *et al.* demonstrated that anti-HCC cytotoxicity of NK cells was improved in HCC cell line exposed to 5 µmol/L of Sorafenib for 48 hours (48) which suggests potential for combined benefits of chemotherapy and immunotherapy (49). Other clinical trials have been conducted to evaluate the efficacy and safety profile of the NK cells in HCC patients and are

schematized in *Table 1*. The outcome of these clinical studies will reveal better understanding of the immunotherapeutic mechanism of NK cell-based therapy for HCC and further advancement of the clinical strategies.

IRE ablation

Surgical resection and partial transplantation are preferred curative options for patients with HCC. However, they are directly affected by several factors including but not limited to tumor location and stage, patient condition, and liver functions. Therefore, a limited number of patients are suitable for these treatment approaches. The local ablation techniques, using thermal or chemical energy, have been developed in the last three decades and demonstrated benefits for the patients who are not suitable surgical candidates (3,54). In addition, ablation methods became a recommended treatment approach for very early (tumor diameter <2 cm) and early-stage (tumor diameter <3 cm) HCC following the meeting of the European Association for the Study of the Liver in 2017 (3,55). RFA, first utilized in the 1990s, became the first line of ablation methods, later followed by MWA and cryoablation. Briefly, RFA utilizes thermal energy delivered to targeted lesions through radiofrequency electrodes placed under imaging guidance to destroy malignant tissues. During RFA, electric current leads to necrosis and cell death while high temperature (60–100 ℃) (56,57) limits the efficacy due to local tissue charring and heat-sink effects (56). To overcome these limitations, it was suggested to cool the electrodes internally and use multiple electrodes in the bipolar mode, despite the increased risk of bleeding and adjacent organ damages. In MWA, the heat generated by microwave energy is utilized to destroy targeted tissues similarly to RFA, but with fewer limitations (58). The design of the microwave antenna can elicit even heat transfer within the targeted region. Nevertheless, MWA has not replaced RFA despite proven benefits in comparison studies (59-61). Cryoablation, eliciting tumor necrosis, and cell death through freezing, is susceptible to the cool sink effect which limits its efficacy (57,62). The limitations of these methods derived the clinical need for implementation of a nonthermal source for ablation of the tissue while eliminating heat-sink effect and allowing safe utilization of the method closer to large vessels.

IRE, a non-thermal and minimally invasive ablation method, is a relatively new ablation method that incorporates delivery of high-frequency electrical energy in short pulses to malignant tissues (63,64). IRE leads to abnormal transmembrane electrical potential which increases the permeability of the cell membrane and causes irreversibly open plasma membranes leading to apoptosis (65-67). Compared to the alternatives, destruction of the cells without thermal features enables the potential application of IRE procedure without damaging close tissues, e.g., vessels, nerves, or ducts (12). Moreover, boundary between treated and untreated tissue region after the procedure can lead to a better evaluation of the treatment response by allowing easier monitoring and controlling the procedure (68). The reversible electroporation (RE) and IRE zones generated by IRE ablation can be visualized in which IRE zone includes dead cells and RE zone represents the less affected peripheral region (*Figure 3*). In magnetic resonance imaging (MRI), IRE zone was observed as hyperintense on T1w and T2w images due to the coagulative necrosis while RE zone is described as hypointense on T1w and hyperintense T2w MRI images (69).

The safety profile of the IRE ablation methods has been investigated by several studies (70-76). Dollinger *et al.* analyzed the effects of IRE on vascular structures by examining subacute (1 and 3 days) and midterm (average 5.7 months) follow-up data (70). In their study, vascular changes were detected in 19 vessels among 194 venous structures located in a 1 cm distance ablation zone in which IRE was performed on 84 hepatic lesions. The follow-up investigation demonstrated partial or complete vascular recovery on 9 of 14 cases, concluding that vascular structures located close to the IRE ablation zone were lightly affected. Another study investigated the injury of bile ducts following IRE procedure in which 55 bile ducts were examined within a 1 cm distance of 53 hepatic tumors (71). The follow-up MRI analyses showed that 8 of 55 bile ducts had a narrowing, 7 had dilation while no adverse events were observed on the remaining 40 bile ducts. In addition, a recent review showed that IRE is a safe alternative to perform at the tissues close to the critical structures with a lower risk of collateral damage compared to other ablation methods (37).

The efficacy of IRE was reported in studies using different animal models (12,77-80). Guo *et al.* performed IRE on thirty Sprague-Dawley rats with HCC and used MRI for evaluation of the therapeutic response 14 days after completion of the procedure (77). Their results suggested that IRE is an efficient method to ablate liver tumor tissue as a potential candidate for HCC treatment. Lee *et al.* assessed the effectiveness of

Table 1 The list of clinical trials registered to United States National Library of Medicine (www.clinicaltrials.gov) for treatment of HCC patients using NK cells

Study title	Phase	Intervention/treatment	Patient	Status	Results	Identifier
Safety study of NK cells from sibship to treat the recurrence of HCC after liver transplantation	1	Bio.: low dose NK cells × 4 times; normal dose NK cells × 4 times; normal dose NK cells × 8 times	18	U	No results posted	NCT02399735
Safety and efficacy of allogeneic NK cells therapy in patients with advanced HCC	1; 2	Bio.: allogeneic NK cells therapy	200	R	No results posted	NCT04162158
By using adoptive transfer of autologous NK cells to prevent recurrence of HCC after curative therapy	2	Bio.: NK cells; Other: curative therapy	140	U	No results posted	NCT02725996
A novel immunotherapy for liver transplant patients with HCC: anti-tumor effect of IL2-activated donor liver NK cell	1	Bio.: liver NK cell inoculation	10	C	No study related adverse effects observed	NCT01147380
A single center, open-label, phase II clinical trial to evaluate the efficacy and safety of MG4101 (*ex vivo* expanded allogeneic NK cell) in HCC after curative resection	2	Bio.: MG4101	5	C	No results posted	NCT02008929
Multi-center, open, phase 2a clinical trial to evaluate the efficacy and safety of MG4101 in HCC after TACE	2	Bio.: MG4101	78	C	No results posted	NCT02854839
A randomized controlled study of cytokine-induced killer cell (CIK) treatment in patients with HCC who underwent radical resection.	3	Bio.: CIK cell treatment	200	C	Median TTR: 13.6 (CIK); 7.8 (control) No significant DFS or OS difference Stage I/II adverse events	NCT00769106
A phase I study to evaluate the safety and efficacy of autologous immune killer cells (IKC) in patients with late-stage HCC or lung cancer	1	Bio.: IKC	20	C	No results posted	NCT03515252
A Phase II/III clinical trial with *ex vivo* expanded autologous immune killer cells to treat liver cancer patients as an adjunct therapy	2; 3	Bio.: IKC; Proc.: TACE	60	R	No results posted	NCT03592706
A randomized controlled study of CIK cell treatment in patients with HCC who underwent radical resection	3	Bio.: CIK cells	200	C	No results posted	NCT01749865
Long-term, non-interventional, observational study following treatment with fate therapeutics FT500 cellular immunotherapy	NA	Gen.: allogeneic NK cell	76	R	No results posted	NCT04106167
Evaluation of CIK cells as therapy or adjuvant treatment for patients with advanced HCC	3	Bio.: CIK; Proc.: TACE	20	U	No results posted	NCT02568748

Table 1 (*continued*)

Table 1 (*continued*)

Study title	Phase	Intervention/treatment	Patient	Status	Results	Identifier
Study evaluating the efficacy and safety of chimeric antigen receptor (CAR) modified pNK cells in MUC1 positive advanced refractory or relapsed solid tumor	1 & 2	Bio.: anti-MUC1 CAR-pNK cells	10	U	No results posted	NCT02839954
FT500 as monotherapy and in combination with immune checkpoint inhibitors in subjects with advanced solid tumors (phase I)	1	Drug: FT500, Nivolumab, Pembrolizumab, Atezolizumab, Cyclophosphamide, Fludarabine	76	R	No results posted	NCT03841110
Phase I/II study of NK T cell infusion in patients with advanced solid tumor	1 & 2	Bio.: NK T cell	120	U	No results posted	NCT02562963
FATE-NK100 as monotherapy and in combination with monoclonal antibody in subjects with advanced solid tumors	1	Drug: FATE-NK100, Cetuximab, Trastuzumab	100	A/NR	No results posted	NCT03319459
A phase I open label, single site, safety and efficacy study of the effects of autologous NK and NK T cell immunotherapy on malignant disease	1	Bio.: autologous NK/NK-T cell immunotherapy	24	S	No results posted	NCT00909558

A, active; C, completed; NR, not recruiting; R, recruiting; S, suspended; U, unknown; HCC, hepatocellular carcinoma; NK, natural killer; TACE, transarterial chemoembolization; DFS, disease-free survival; OS, overall survival.

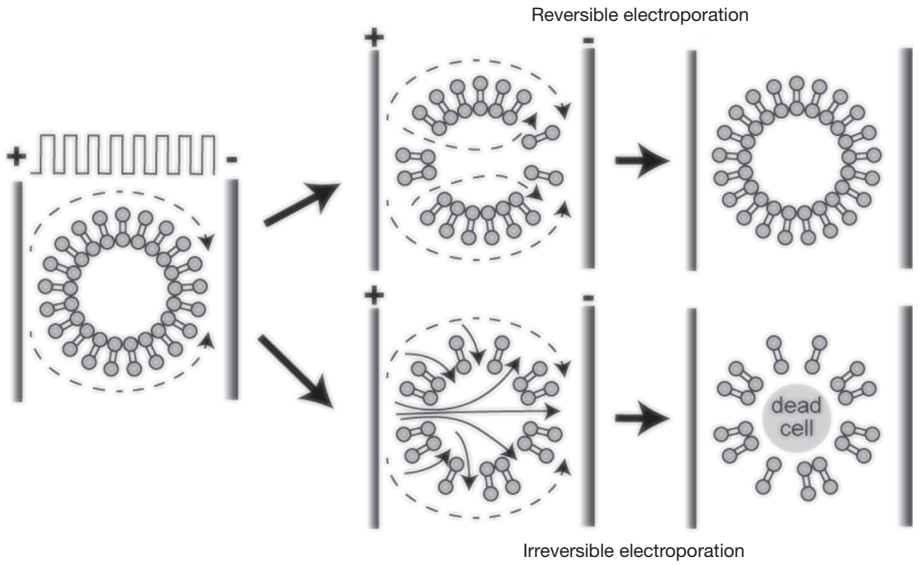

Figure 3 The representation of irreversibly and reversible electroporated zones after the irreversible electroporation (IRE) ablation procedure. Complete cell death occurs within the irreversible electroporation zone while cells in the peripheral region are minimally affected and demonstrate recovery after IRE ablation.

the IRE ablation by performing a procedure on healthy liver tissue of sixteen Yorkshire pigs (12). They did not observe any complications following the IRE procedure for 55 ablation zones of 16 subjects which suggested IRE as a safe and effective ablation approach for necrosis. In another experiment, potential clinical translation of the IRE ablation method was investigated using VX2 liver tumor model with single or multiple ablations on twenty New Zealand white rabbits (80). Several studies focused on immediate therapeutic response following IRE in animal models (78,81-87). Zhang *et al.* investigated the changes using MRI after IRE with different voltages in a rat liver model (78). Additional studies demonstrated that other imaging modalities (ultrasound, contrast-enhanced computed tomography (CT), diffusion-weighted MRI, and contrast-enhanced MRI) have the potential for early assessment of IRE treatment effects by visualizing immediate changes following the ablation (81-85). More recently, an advanced MRI technique was used on VX2 rabbit HCC model to differentiate IRE regions from reversibly electroporated (RE) zones which will be beneficial for intraprocedural assessment (86,87). The results further emphasize the potential of immediate evaluation of RE regions using MRI to determine the treatment strategy (*Figure 4*).

The clinical outcome of IRE ablation in patients with solid tumors has also been performed (73,88-98). Thomson *et al.* investigated the safety of the IRE technique in a single-center prospective non-randomized study including 38 patients with advanced liver, kidney, or lung malignancies and unresponsive to alternative therapies (73). The results demonstrated that IRE is a safe ablation method in clinical usage for cancer treatment. Cannon *et al.* performed a clinical investigation to evaluate the efficacy of IRE on hepatic tumors (88). Of 44 patients undergoing IRE treatment, five patients had 9 adverse effects which were classified as unrelated (leukocytosis, urinary tract infection), indirectly related (dehydration, biliary stent occlusion, cholangitis, and acute renal failure), procedure-related (neurogenic bladder, abdominal pain, and flank pain) and all these effects were resolved in 30 days. Moreover, the study results completed by Sugimoto *et al.* suggested that IRE was well tolerated by the patients with small tumor size and satisfactorily controlled disease (91). Eller *et al.* performed the IRE procedure on 14 patients who were not surgical candidates and had lesions near large vessels (92). Ten of the 14 patients were successfully treated without local recurrence at least for a mean of 388±160 days. In addition, Niessen *et al.* discussed that patients with larger tumor volume may be poor candidates for IRE treatment due to the association of tumor volume and early disease recurrence observed in their study (93). More recent studies also demonstrated that IRE is a safe and efficient alternative ablation method for patients with unresectable tumors (96-98).

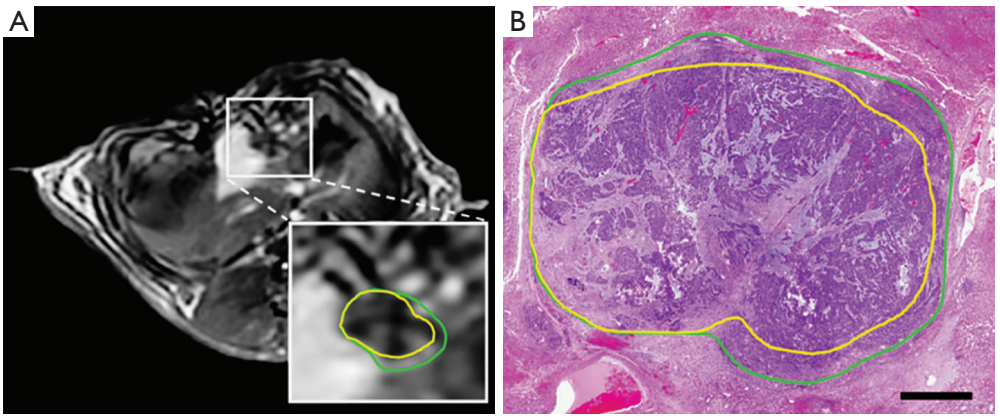

Figure 4 Magnetic resonance imaging (MRI) and histology-based irreversible electroporation (IRE) and reversible electroporation (RE) areas. (A) Representative postcontrast T1-weighted MRI where the reversibly electroporated zone is outlined in yellow and the peripheral reversibly electroporated zone in green. (B) H&E-stained histology slide, corresponding regions were demonstrated in which the necrotic (irreversible) center is marked in yellow and the enhanced rim is emphasized in green. [Adapted from Figini *et al.* (87). Copyright 2018 by John Wiley & Sons Ltd.].

Currently, there are completed and ongoing clinical trials for the assessment of efficacy and feasibility of the IRE ablation method in the treatment of HCC (*Table 2*). The comprehensive evaluation of the IRE procedure during these clinical trials will further present evidence for efficacy and safety-profile of IRE as well as advance our knowledge of the tumor response following ablation.

IRE plus NK cell-based treatment

Previous studies have demonstrated the clinical benefits of the IRE ablation procedure by highlighting advantages compared to thermal ablation methods including a high ablation rate and recurrence-free disease period not affected by blood flow (10,64,88,89,99,100). However, several studies have demonstrated possible advancements for this local ablation method for the efficient treatment of cancer patients (101-104). Particularly, a preclinical study completed by Neal *et al.* suggested that immunocompetent subjects may have a better IRE therapeutic response compared to immunodeficient subjects (105). Therefore, boosting the immune system of the candidate patients remains important for improved IRE treatment outcomes. As an essential element of the innate immune system, NK cells are one of the most promising therapeutic agents to perform following IRE ablation for the treatment of solid tumors. Lin *et al.* investigated the safety and clinical efficacy of this potential combinative approach for the treatment of advanced-stage pancreatic cancer patients with unresectable tumors (106). Throughout this clinical trial, patients had local or systemic adverse effects measured as grade 1 (nausea andhaoba emesis, 7.04%; puncture point pain, 29.58%; fatigue, 22.54%, fever, 30.99%) or grade 2 (duodenum and gastric retention, 4.23%, transient reduction of intraoperative blood pressure, 25.35%; white cell count reduction, 18.31%), and all of these side effects were resolved on the same day with symptomatic treatments without any complications. In addition, median progression-free survival and overall survival of the patients treated with a combination of IRE ablation and NK cell-based immunotherapy were significantly improved in stage III pancreatic cancer patients as well as median overall survival of stage IV patients was extended. The clinical efficacy of combination treatment for stage IV HCC was initially reported by Alnaggar *et al.* (45). In this study, twenty patients only received an IRE ablation while the remaining twenty patients additionally received NK cell-based immunotherapy 4–6 days after IRE ablation. The procedures were completed without severe complications and patients with mild adverse effects treated with symptomatic management. The study showed that patient cohort that received combination treatment had significantly improved median overall survival (10.1 months) compared to patients treated with a single therapy (8.9 months, P=0.0078). Moreover, observation data acquired 3 months following the treatments demonstrated a significant difference in tumor size among the groups (IRE: 2.68±1.01 *vs.* NK-IRE: 2.31±0.68 P<0.05), and combination group obtained a higher disease control rate

Table 2 The list of clinical trials registered to United States National Library of Medicine (www.clinicaltrials.gov) for treatment of HCC patients using IRE

Study title	Phase	Intervention/treatment	Patient	Status	Results	Identifier
A prospective, multi-center, clinical trial using IRE for the treatment of early-stage HCC	NA	Dev.: NanoKnife Low Energy Direct Current (LEDC) System	26	C	77% CR presented on 1 month biopsy-proven results	NCT01078415
A prospective clinical trial using IRE for the treatment of unresectable hepatic carcinoma (HC) close to the gallbladder	NA	Dev.: NanoKnife	30	R	No results posted	NCT02332551
HC in poor liver function: safety and efficacy of IRE	NA	Dev.: NanoKnife LEDC System	15	R	Frequent hypertension due to adrenal gland damage	NCT02352935
Comparison of immunological response after MWA and IRE of HCC	NA	Device: MWA, NanoKnife IRE	40	W	No results posted	NCT03040453
A prospective clinical trial using IRE for treatment of liver cancers	NA	Dev.: IRE System	18	C	No results posted	NCT02828865
Study of the contribution of automatic image fusion of a cone-beam CT volume with ultrasound during percutaneous ablation treatment of hepatic tumors	NA	Rad.: interact active tracker	60	R	No results posted	NCT04420026
IRE of unresectable liver tumors - a phase I study of safety and feasibility	NA	Proc.: IRE	15	R	No results posted	NCT04404647
Prospective evaluation of tumor response to cancer treatment therapies	–	Proc.: TACE, Y-90, MWA, IRE	10	T	No results posted	NCT02787954
Evaluation of tumor ablation effects by IRE for patients with malignant liver tumors	NA	Proc.: IRE	20	C	No results posted	NCT02010801

A, active; C, completed; R, recruiting; T, terminated; W, withdrawn; HCC, hepatocellular carcinoma; IRE, irreversible electroporation; MWA, microwave ablation; NA, not available; TACE, transarterial chemoembolization.

than IRE group. A recent comprehensive study reported the safety and short-term efficacy of the IRE plus NK cell-based immunotherapy for the treatment with unresectable primary liver cancer patients enrolled in the first clinical trial (NCT03008343) in collaboration with Fuda Cancer Hospital and Shenzhen Hank Bioengineering Institute (107). The results demonstrated that performing NK cell-based immunotherapy following IRE ablation generates a synergistic effect that significantly reduces the number of tumor cells in circulation and improved the immune functions of the patients. The researchers stated that 88.9% of the patients treated with the combination therapy demonstrated clinical response while 68.2% of the patients treated with only IRE ablation in three months post-treatment. Moreover, significantly improved progression-free and overall survival was observed for the patients receiving combination treatment.

Conclusions

During the past few decades, many studies have demonstrated the potential benefits of immunotherapy for the treatment of solid tumors. As a key agent of the innate immune system, NK cells have great promise for fighting against tumor cells. Adoptive transfer of NK cells has further emphasized the potential benefits for cancer patients with solid tumors. However, immunosuppressive tumor microenvironment and inadequate homing efficiency of NK cells to tumor tissues (particularly following systemic administration) have inhibited ATI efficacy. As a minimally invasive non-thermal ablation technique, IRE instigates cell death within the applied region with minimal damage to the perpendicular region. The destruction of the tumor microenvironment by IRE enables overcoming tumor immunosuppression. Recent studies have demonstrated that a combination of IRE and NK cell-based immunotherapy has the potential to improve patient survival in the advanced-stage liver and pancreatic cancers. Besides, several studies that combine other immunotherapeutic methods with IRE ablation method have presented promising outcomes for the treatment of pancreatic tumors in which comprehensive reports will be beneficial for the researchers with the focus of cancer research (108-112). The data to be acquired from future studies will further emphasize the benefits of utilizing combination treatment of patients with HCC and also support the findings for assessment of long-term clinical response.

Acknowledgments

Funding: This study was supported by the National Cancer Institute (grants R01CA196967, R01CA209886, and R01CA241532), 2019 Harold E. Eisenberg Foundation Scholar Award, and SIR Foundation Pilot Grant (PR-0000000012).

Footnote

Reporting Checklist: The authors have completed the Narrative Review reporting checklist. Available at https://dx.doi.org/10.21037/atm-21-539

Peer Review File: Available at https://dx.doi.org/10.21037/atm-21-539

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (Available at https://dx.doi.org/10.21037/atm-21-539). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Eresen A, Yang J, Scotti A, Cai K, Yaghmai V, Zhang Z. Combination of natural killer cell-based immunotherapy and irreversible electroporation for the treatment of hepatocellular carcinoma. Ann Transl Med 2021;9(13):1089. doi: 10.21037/atm-21-539

