Review

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Combined locoregional-immunotherapy for liver cancer

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Summary

Locoregional therapies are commonly used to treat patients with hepatocellular carcinoma. It has been noted for many years that locoregional therapies may have additional systemic effects other than simple tumour elimination. Immunological "side effects" have been described in response to locoregional therapies in animal studies and in patients. With the advent of immunotherapy for hepatocellular carcinoma, there is increasing interest in determining the best way to combine immunotherapy with locoregional therapies. Herein, we provide a compact summary of answered and unanswered questions in the field, including: What animal model is best suited to test combined immune-locoregional treatments? How does tumour cell death affect immune responses? What type of immune responses have been observed in patients treated with different types of locoregional therapies? What can be surmised from the results of the first study testing the combination of locoregional therapy with immune checkpoint blockade? Finally, we discuss the outlook for this rapidly growing area of research, focussing on the issues which must be overcome to bridge the gap between interventional radiology and cancer immunology.

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Animal models to study locoregional therapies

Animal models enable preclinical optimization and delivery of novel experimental drugs and immunomodulators prior to human trials. Local, intra-arterial or intratumoral, delivery of new agents with potentially lethal toxicity when administered systemically, should be tested in a preclinical paradigm first. In preclinical survival models, longitudinal and multiple surrogate and mechanistic biomarkers can be sampled, including tumour, blood, and repeated imaging over time, without concerns relating to radiation exposure.

Multiple methods exist to induce tumour formation in mice, including genetically engineered mouse models, chemotaxis agents, intrahepatic or intrasplenic injection of tumour cells and xenograft approaches. Additionally, as hepatocellular carcinoma (HCC) generally develops in the context of a diseased liver, methods exist to induce liver disease in mice to mimic viral hepatitis, fatty liver disease, fibrosis, alcohol-induced liver disease and cholestasis.[1] Furthermore, humanized mice may potentially mimic the human immune response, however this technique is challenging and this approach has not been adopted by researchers in the field of HCC immunotherapy to date.

Most of our understanding of the mechanism of the immune system in liver cancer has been drawn from experiments in murine models. The mouse model has throughput advantages, allowing treatment of a large number of animals with easy handling, housing and lower costs. However, their size becomes a geometric disadvantage for investigation of local therapies and drug delivery, due to the disparities in scale. Researchers are limited in their ability to model and apply locoregional techniques. Ablations of subcutaneous tumours can only be performed on rather small and spherically shaped tumours in order to prevent skin burn and damage to surrounding tissues, such as nerves, which can compromise the animals. Intra-arterial drug delivery is highly challenging in murine and rat models due to small vessel calibre. Both access vessels and target vessels within the liver are smaller than standard clinical devices and invasive imaging equipment. Specific microsphere or bead sizes may have very different biologic effects in such small vessels, compared to human or large animal vessels, purely because of differences in geometry and scale. Furthermore, contrast agents that can be used in real time to guide intra-arterial procedures in mice models are lacking. Contrast agents used in humans are non-ionic and water soluble and therefore cleared from mice blood too rapidly to be used intra-procedurally with high resolution imaging. IV ultrasound contrast agents have not met the requirement for arterial phase information.

At least 3 animal models that are used to investigate the effects of interventional oncology tools on liver cancer are of a sufficient size to model locoregional therapy. The rabbit VX2 model has been widely used for various ablation techniques and intra-arterial drug delivery to hepatic tumours.[2–4] However, this model has a few major limitations for immunological investigations. First,

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Key points

There are a number of advantages of mice models, however their size is a geometric limitation when investigating local therapies and drug delivery.

Key points

A carcinogen-induced woodchuck HCC model is more appropriate for immune investigations and can be used to assess locoregional therapies, new therapeutics and interventional oncology paradigms.

VX2 is a cell line classified as leporine anaplastic squamous cell carcinoma induced by papilloma virus, which does not resemble most human HCC subtypes histologically and genetically.[5] The VX2 model is an orthotopic animal model with technical and resource challenges. Cells are propagated in the muscle of one donor animal and monitored until a sufficient tumour volume is reached, at which point the tumour is excised, the necrotic part is removed, and only the viable tumour is transplanted into a recipient animal. These VX2 cells can be implanted in various locations such as the flank, liver, lung and kidney.[6–8] The transplantation technique leads to the second limitation of this model for immunology investigations. The donor and the recipient are not genetically identical; thus, the tumour can be considered an allograft, rather than an autograft. Therefore, it is possible that the recipient animal recognizes the tumour as non-self and rejects the tumour or alters the immune responses. Third, the removal of the necrotic part of the tumour before implantation might confound the investigation of the immune response, or delete antigens or immune elements. Lastly, implanted VX2 tumours are unlikely to recapitulate the human HCC microenvironment. VX2 hepatic tumours evolve and grow with peripheral vascularity, developing central or heterogeneous necrosis, likely because the central zone outgrows its blood supply. This might hamper antigen recognition or presentation, or subsequent influx of immune cells to the tumour, potentially confounding not only drug efficacy investigations but also limiting our understanding of the immunomodulation after local or regional therapy. The heterogeneity of VX2 tumours, unlike human tumours, is somewhat limited and as a result, pathways of immune activation or escape may be absent, poorly modelled, or overlooked. Two autochthonous HCC models, rat and woodchuck, were introduced in recent years for investigation of interventional oncology paradigms.[9,10] HCC is induced in the former by the toxin diethylnitrosamine (DEN) and in the latter by woodchuck hepatitis virus. In both models, as in most patients, the tumours develop spontaneously in the liver on the background of liver disease: fibrotic liver or chronic inflammation, respectively. The degree of recapitulation of associated cirrhosis may vary. Autochthonous models might be superior to orthotopic models as they take into account the immunological particularities of the liver, an organ where both induction of tolerance and effective response against pathogens, probiotics and food-derived antigens must take place.[11] A meta-analysis, even though performed on colon cancer models and tissues, found that among animal models, carcinogen-induced tumours had the best correlation with clinical responses.[12] Among these 2 carcinogen-induced HCC models, it is possible that the woodchuck model is more appropriate for immune investigations, as analysis of the mutational landscape in different murine HCCs has shown that DEN tumours are the least similar to human disease and almost universally carry the Braf V637E mutation, which is rarely found in human HCC.[13]

The gradual growth of tumours within the liver may result in better modelling, with the development of an adherent and hypertrophied arterial blood supply which mimics human HCC and may facilitate better interactions with the immune system. The woodchuck HCC model develops large, hyper-vascularized tumours that can be selectively targeted (Fig. 1). This model can be utilized to assess locoregional therapies, new therapeutics and interventional oncology paradigms; tumour heterogeneity and genetic variation should be further investigated in this model but may offer the most appropriate conditions for the study of novel therapeutics for HCC (Fig. 2).

Cell death and antitumour immunity

The question of how cell death shapes tumour immunity has been studied widely.[14] There are 3 main immunological mechanisms affected by cell death: tumour antigens, antigen presenting cells, and effector cells. Preclinical studies have added a lot to our understanding of how the immune system may react to locoregional therapies and subsequent cell death. It is a well-known fact that the type of cell death has significant effects on antitumour immunity. Using a very clean, but artificial

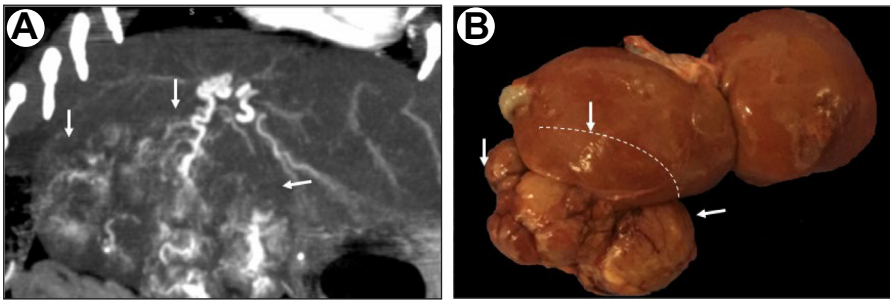

Fig. 1. Spontaneous HCC developing in chronically hepatitis infected woodchucks. (A) Contrast-enhanced CT scan shows large heterogenous tumour with robust arterial blood supply (B). Gross pathology of liver and tumour (tumour edges demarcated by white arrows, margins of the tumour behind the liver demarcated by dashed line). HCC, hepatocellular carcinoma.

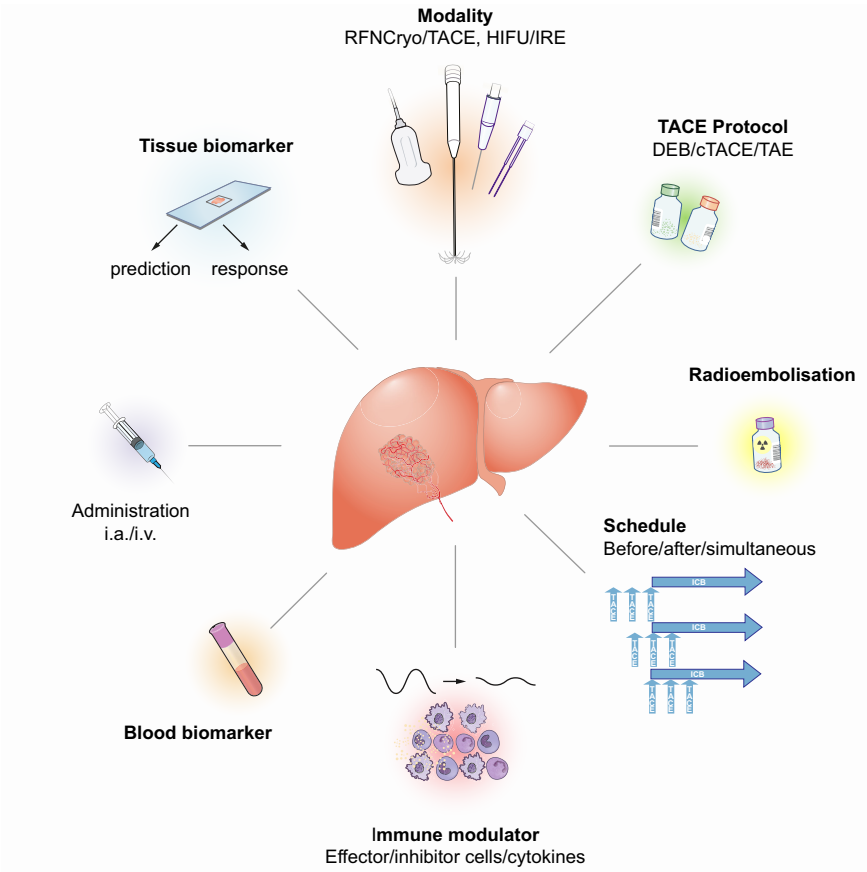

Fig. 2. Overview of considerations for future research studies combining immunotherapy with locoregional therapies. cTACE, conventional TACE; HIFU, high-intensity focussed ultrasound; IRE, irreversible electroporation; RFA, radiofrequency ablation; TACE, transarterial chemoembolization; TAE, transarterial embolization.

ex vivo model in which we killed tumour cells prior to injection using 3 freeze–thaw cycles (cryoablation) or gamma irradiation, we demonstrated that apoptotic, but not necrotic tumour cell death, induced tumour-specific immunity.[15] Interestingly, we showed that via pathogen associated molecular pathway using DNA immune modulating agents such as p(dI-dc) and/or unmethylated CpG DNA, an immune response to tumour cell lysates was induced.[16] Additional molecular studies revealed that necrotic tumour cell death failed to induce antitumour immunity due to the activity of an oligopeptidase in freeze-thawed cells.[17] The presence and processing mechanisms of antigens that initiate antitumour immune responses are likely governed by the type of cell death or cell injury, as well as the supply of requisite molecular chaperones, antigen-presenting cells, regulatory T cells, and proximity of nearby blood vessel supply chain. Such a complex interactive dance of dynamic molecular processes is challenging to characterize with the simple descriptive rules of linear cause and effect. Animal models fall short of recapitulating the complex and dynamically evolving immunologic processes and pharmacological immunomodulation observed in human HCC.

Obviously, the situation is more complicated when cell death occurs *in vivo*. The group of Zitvogel and Kromer intensively studied the effect of chemotherapy induced cell death and its effect on antitumour immunity. They showed that the initiating stimulus can cause an immunogenic or non-immunogenic cell death. Immunogenic cell death (ICD) involves the release of calreticulin and other endoplasmic reticulum proteins at the cell surface, the secretion of ATP during the blebbing phase of apoptosis, and the cell death-associated release of the non-histone chromatin protein high-mobility group box 1 (HMGB1), facilitating recruitment and activation of dendritic cells into the tumour microenvironment, which will allow engulfment of tumour antigens from dying tumour cells and optimal antigen presentation to T cells.[18] Chemotherapeutic reagents known to induce and potentiate ICD include doxorubicin and oxaliplatin, while cisplatin fails to induce ICD.[19] Doxorubicin is the most commonly administered drug in HCC chemoembolization (transarterial chemoembolization [TACE] or drug-eluting bead TACE [DEB-TACE]), which may have broad implications for its use in image guided regional therapies for HCC.

The type of cell death not only determines immune effects, but also the type of tumour in experimental mouse models. It was recently reported by the Zender lab that an apoptosis-associated hepatic cytokine microenvironment determines HCC outgrowth from oncogenically transformed hepatocytes, whereas hepatocytes containing identical oncogenic drivers give rise to intrahepatic cholangiocarcinoma (ICC) if they develop in a necroptosis-associated microenvironment. Pharmacological or genetic suppression of necroptosis reverses the necroptosis-dependent cytokine microenvironment and switches ICC to HCC.[20]

In 2003, one of the first studies focussing on the effect of locoregional therapies on antitumour immunity reported an activation of tumour-specific T cells upon radiofrequency ablation (RFA) in a VX2 carcinoma model in rabbits.[21] Radiofrequency and cryoablation have been shown to provide an antigen source for dendritic cells *in vivo*.[22] In our own studies, subtotal RFA resulted in enhanced tumour-specific immune responses *in vivo* and an infiltration of tumours by dendritic cells.[23] These mechanisms function mainly by upregulating antigen presentation. The effects of thermal ablation (percutaneous RFA, microwave ablation, cryoablation and irreversible electroporation) on immune responses are the subject of an insightful, although somewhat speculative, recent review.[24]

Finally, different immune-based approaches have been evaluated in combination with locoregional therapies in animal models. Using a well-defined murine B16-ovalbumin (B16-OVA) tumour model, the CTLA4-blocking antibody was one of the first compounds to be tested in this setting. The authors demonstrated not only a weak but detectable immune response directed against OVA, but also against a broader range of B16 antigens. The significance of this effect was confirmed in adoptive transfer experiments, in which splenocytes derived from mice after RFA ablation were transferred into naïve mice challenged with live tumour cells. Anti-CTLA4 treatment at the time of tumour destruction enhanced antitumour immunity.[25] Similar results were observed in a preclinical murine prostate cancer model. Anti-CTLA4 treatment augmented the effect of cryoablation leading to impaired growth of secondary tumours.[26] The combination of RFA and TLR9 stimulation was tested in a VX2 hepatoma model and the combination was shown to prevent subsequent tumour spread.[27] Similar results have been obtained in a B16-OVA model. Combination treatment with cryoablation plus TLR9 stimulation via CpG-oligodeoxynucleotides was very effective in the eradication of local and systemic tumours due to enhanced dendritic cell maturation leading to more efficient cross-presentation in tumour-bearing mice.[28]

Key points

Ablation and embolization techniques have been shown to induce immune responses in patients with HCC.

Photodynamic therapy (PDT) uses non-toxic photosensitizers and harmless visible light in combination with oxygen to produce cytotoxic reactive oxygen species that selectively kill malignant cells by apoptosis and/or necrosis and shut down the tumour microvasculature, while potentially sparing normal tissue.[29] It has been shown to cause acute inflammatory responses, expression of heat-shock proteins and immune cell infiltration, thereby inducing an ICD. PDT has been shown to prevent outgrowth of distant untreated tumours and prevent mice from tumour re-challenge in a CD8+ T cell dependent manner. Anti-CTLA4 treatment significantly improved therapeutic efficacy and survival of mice.[30] Similar results have been observed in combination with imiquimod (R837), a Toll-like-receptor-7 agonist, when co-encapsulated into a nanoparticle with the photothermal agent, indocyanine green, by poly(lactic-co-glycolic) acid (PLGA). Treating mice with this nanoparticle in combination with anti-CTLA4 generated strong immunological responses capable of inhibiting metastasis formation and leading to tumour shrinkage.[31] IL-2 treatment in the form of a fusion protein bound to a tumour-specific antibody was shown to enhance antitumour immunity against colon tumours treated with RFA.[32]

Insufficient RFA has also been shown to promote angiogenesis of residual HCC[33] and promote tumour growth of non-small cell lung cancer cells[34] via HIF-1α/VEGFA. Subtotal ablation can also induce hepatic regeneration and tumorigenesis via IL-6, c-met, or HGF-dependent pathways.[35] Nonetheless modulation of RF heating parameters alone or in combination with adjuvant heat-shock proteins inhibition, STAT3 inhibition, or simple cyclooxygenase-2 selective non-steroidal anti-inflammatory drugs can mitigate or reduce unwanted, off-target systemic tumorigenic effects.[36] Nevertheless, there may be a precarious and poorly understood balance between partial ablation or embolization inducing a tumorigenic response *vs.* an immune regression. Tipping this balance via pharmacomodulation may more fully recognize the preclinical promise and augmentation of HCC immunomodulation combined with local and regional image guided tools.

Immunological effects observed in patients treated with locoregional therapies

Locoregional therapy has been shown to induce immune responses in patients with HCC.[37] No direct comparison between the different modes of ablation has been conducted and reports primarily focus on individual modalities or at best 2 modalities. The effect of percutaneous microwave ablation (MWA) as a single therapy was one of the first to be investigated. Recently, immune responses (T cell activation and IL-12 release)

4 weeks after treatment have also been reported.[38,39] RFA is another ablation technique more commonly used to treat intrahepatic HCC. The hypothesis that ablation can cause tumour-specific immune responses was tested on tumour samples and peripheral blood mononuclear cells (PBMCs) collected from patients before and after RFA.[40] HCC specific T cell expansion was measured *in vitro*. It was shown that HCC specific T cell responses were induced by stimulation with the thermally ablated HCC extract. Using an antigen library, it was shown that up to 60% of the patients responded with T cell activation against at least one antigen after RFA in another study.[41] It was found that a higher number of antigen specific CD8+ T cells after RFA correlated significantly with the length of recurrence-free survival in patients with HCC. Another important question is whether and how locoregional therapies may influence immunosuppressor mechanisms. Myeloid-derived suppressor cells (MDSCs) are known as key immune regulators in various human malignancies, and it was reported that $CD14^{+}HLA\text{-}DR^{-/low}$ are increased in patients with HCC, where they inhibit the function of T cells through the induction of regulatory T cells.[42] In a trial aimed at investigating the ratio between MDSCs and CD14 cells in patients with HCC, it was found that this ratio was significantly increased compared to healthy donors and patients with chronic hepatitis, and that the frequency of MDSCs correlated with tumour progression.[43] In patients who received RFA, the frequency of MDSCs was significantly decreased after treatment. However, in several patients the frequencies were increased after RFA and patients with a high frequency of MDSCs after treatment relapsed. Similar results have been described by others.[41] It was also suggested that ablation therapy alters the T cell balance by increasing the systemic ratio of cytotoxic T lymphocytes to regulatory T cells. Heat-based ablation (RFA and MWA) might be a more effective approach than cryoablation to enhance systemic antitumour immunity.[44] MDSCs and regulatory T cells in raw numbers, and not only their balance among T cells may influence immune responses, as well as their measurements in tumour, nodes or peripherally, this further complicating their predictive value.

Like ablation, transarterial chemoembolization (TACE) and drug-eluting bead TACE (DEB-TACE) cause local cell death. Doxorubicin is the preferred and most common chemotherapeutic agent used in TACE and it is considered an immunogenic drug, potentiating ICD.[45] Tumours treated by TACE are usually larger than those treated by ablation and it is not clear how that affects immune responses, nor is it known whether a subtotal treatment is ideal providing that there is not overwhelming tumour burden and a vascularized route to the remaining viable tumour. TACE was shown to promote an ICD[46] and to induce tumour-associated antigen specific responses.[47] Glypican-3 (GPC3) is a carcinoembryonic antigen and an ideal target for anticancer immunotherapy against HCC.[48] The induction of a GPC3 specific T cell-mediated immune response was investigated in patients with HCC.[49] It was shown that circulating GPC3-specific cytotoxic T lymphocytes were increased in 55% of patients with HCC after RFA and in 44% of patients after TACE, but in only 11% of patients after surgical resection. To investigate GPC3 expression in HCC tissues, the researchers performed immunohistochemical staining for GPC3 in biopsy or resected specimens. Of note, all 7 patients with GPC3-expressing HCCs exhibited an increase in GPC3-specific cytotoxic T lymphocytes after RFA or TACE, whereas none of the 7 patients with GPC3-expressing HCCs did after surgical resection. The difference in GPC3-specific cytotoxic T lymphocyte activation between pre- and post-treatment in the RFA group was larger than that in the resection group ($p = 0.023$). This difference in the TACE group was also larger than that in the resection group, but the difference was not statistically significant ($p = 0.096$).

Radioembolization using intra-arterial delivery of Y90 is another locoregional approach for the treatment of HCC. The half-life of the Y90 isotope is approximately 64 hours but maximal clinical response, and the effect on the surrounding liver, is only seen 3–6 months after treatment. Chew *et al.* presented the results of in-depth immunophenotyping at a local and systemic level. The authors found higher immune activation in the tumour microenvironment of resected HCC after Y90 downstaging compared to naïve patients. In the Y90 treated patients, analysis of PBMCs before and after treatment showed an increase in TNF-α on both CD4 and CD8 T cells and an increase in antigen-presenting cell percentage. Furthermore, the authors devised a response prediction model using systemic immune biomarkers pre-treatment.[50] Conflicting data suggested that radioembolization causes lymphopenia, reduction of lymphocyte proliferation capacity, and reduction in their ability to produce inflammatory cytokines directly after treatment, these phenomena were observed up to 1 month after treatment.[51] Radioembolization can result in unwanted inflammation, and hypertrophy of non-treated hemiliver. Both Fernandez-Ros *et al.* and Seidensticker *et al.* showed that pro-inflammatory cytokines were increased after Y90 treatment, the latter also suggested that high levels of IL-8 or IL-6 pre-treatment could predict decreased overall survival.[52,53] In summary, results from a number of studies suggest that locoregional therapies cause changes in tumour-specific and innate immune responses. It is very plausible that changes in the local cytokine and/or chemokine milieu, which are difficult to measure in patients, result from locoregional therapies and affect not only effector cell responses, but also

Table 1. Changes in immune response observed in patients treated with locoregional therapies.

Locoregional treatment	Immune outcome measurements	Source of immune measurements tumor (T), peripheral blood (P)	Number of patients	Year of publication	Reference
MWA	Th17, CD3, CD4, CD8, Tregs	P	30 HCC	2018	38
MWA	CD3, CD4, CD8, CD4+ CD25+ Tregs, and CD16+ CD56+ NK	P	45 HCC	2017	39
RFA	APC maturation and function (*in vitro* assay for tissues before and after ablation)	T, P	19	2008	40
RFA	Tumor-associated antigen-derived peptides	P	69 HCC	2013	41
RFA	MDSCs	P	123 HCC patients, 33 received RFA	2013	43
TACE	Cell death sera markers: HMGB1, sRAGE, DNase	P	50 HCC, 71 procedures	2012	46
RFA/ TACE/surgical resection	Glypican-3-specific CTLs	P	9 RFA, 9 TACE, 9 Surgical resections	2012	49
RFA	Th1 (IL-2, TNF-α, IFN-γ), Th2 (IL-4, IL-6, IL-10)	P	26 HCC, 25 healthy	2017	54
RFA	Immune potentiating antigens in the serum, Ficolin-3	P	57 HCC	2018	55
TACE	IL12p70, IFN-γ, IL-17A, IL-2, IL-10, IL-9, IL-22, IL-6, IL-13, IL-4, IL-5, IL-1β, and TNF-α	P	83 HCC, 33 healthy	2013	56
TACE	CD4 (Th1, Th17 and Treg cells), CD8, NK, NKT, IL-2, IL-4, IL-6, IL-10, IL-17A, IFN-γ, and TNF-α	P	28 stage I HCC, 51 stage III, 20 healthy	2013	57
TACE	CD4, CD8, Treg, IL-35	P	47 HCC, 15 healthy	2015	58
Bland embolization	Th1, Th2, Treg	P	5 HCC	2016	59
Cryo+TACE	CD3/CD4, CD4/CD8, NK	P	32 TACE, 31 Cryo, 35 Cryo+TACE	2015	60
Y90	In depth phenotyping	T, P	41	2018	50
Y90	IL-10, IFN-γ, CD3, CD4, CD8, B, NK, CD45R0	P	25	2018	51
Y90	IL-6, IL-8, TNF-α, nitrotyrosine, malondialdehyde	P	14	2014	52
Y90	IL-1, IL-2, IL-4, IL-6, IL-8, TNF-α, IFN-γ	P	12 HCC (total of 34)	2017	53

APC, antigen presenting cell; CTLs, cytotoxic T lymphocytes; HCC, hepatocellular carcinoma; MDSCs, myeloid-derived suppressor cells; MWA, microwave ablation; RFA, radiofrequency ablation; TACE, transarterial chemoembolization.

Key points

In our study patients responded well to the combination of locoregional therapy and the immune-modulator, tremelimumab, which was well tolerated with no dose-limiting toxicity.

immune suppressor mechanisms such as MDSCs. A range of cytokine, chemokine, and inflammatory/damage-associated molecular patterns/cell stress response markers have been described following virtually all of the various ablative modalities. A summary of studies in this topic and results for patients with HCC are summarized in Table 1.

Combined immune checkpoint blockade plus locoregional therapies

Based on the preclinical and clinical studies described earlier, we decided to test the hypothesis that immune checkpoint blockade may enhance immune responses caused by locoregional therapies. Based on an earlier study by Sangro *et al.*, in which he showed that tremelimumab (anti-CTLA4) may lead to clinical and immunological effects in HCC[61] we treated a total of 39 patients with HCC using locoregional therapy plus tremelimumab. We initially enrolled patients who had progressed on sorafenib therapy. A total of 6 doses of tremelimumab were given at 4-weekly intervals, followed by 3-monthly intravenous administrations until off-treatment criteria were met. On day 35, patients received locoregional therapy (ablation or DEB-TACE). An intentionally incomplete RFA or DEB-TACE was performed with the intent to induce and/or augment an antitumour immune response, and to leave viable tumour tissue at the ablation-tumour junction, which may be a fertile and key site for cytokine, inflammatory and immune activity. We also enrolled a subgroup of patients with Barcelona Clinic Liver Cancer stage B HCC who were eligible for TACE. This design was chosen so that patients had recovered from potential tremelimumab-related immune mediated side effects such transaminitis, which had initially been reported to occur in patients with HCC who were treated with tremelimumab.[61] The primary endpoints of this study, feasibility and safety, were met.

After a median follow-up of 36.6 months we observed a median overall survival of 10.9 months (95% CI 8.0–13.7 months). The overall median survival rates in the groups treated with TACE, RFA and cryoablation were 13.8 months (95% CI 10.2–17.4 months, n = 17), 9.2 months (95% CI 6.6–11.2 months, n = 10), 15.0 months (95% CI 10.5–19.5 months, n = 9), respectively. One complete response, 7 partial responses and 15 patients with stable disease were noted among 34 evaluable patients, Aghdashian *et al.* submitted and [62]. Of note, only lesions not treated by locoregional therapy were used to determine tumour responses (see Fig. 3).

Treatment was well tolerated and no dose-limiting toxicity was observed. The most common clinical toxicity was pruritus which was predominantly grade 1 and frequently accompanied by a rash consistent with immune-related dermatitis. Elevated aminotransferases were commonly observed. Other immune-related adverse events included colitis, pneumonitis and endocrinopathies. A comprehensive study of immune corre-

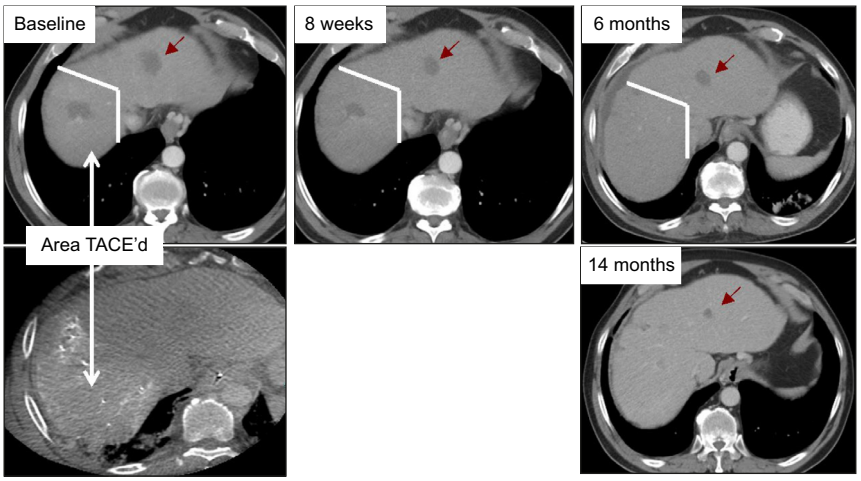

Fig. 3. Tumour response in patient with HCC upon TACE + tremelimumab treatment in lesion not treated by TACE. HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization.

lates in this patient population revealed an influx of T cells into the tumours of patients responding to immunotherapy. Flowcytometry showed an increase in the frequency of $CD4^+$-HLA-DR^+, $CD4^+$-$PD\text{-}1^+$, $CD8^+$HLA-DR^+, $CD8^+PD\text{-}1^+$, $CD4^+ICOS^+$ and $CD8^+ICOS^+$ T cells in the peripheral blood of the treated patients. Patients with higher $CD4^+PD1^+$ cell frequency at baseline were more likely to respond to tremelimumab therapy. Analysis of tumour-specific (alpha-fetoprotein and survivin) T cell responses indicated PD-1 expression was increased upon tremelimumab treatment. An increase of tumour infiltrating $CD3^+$ T cells was also seen in these patients. Immunosequencing of longitudinal PBMCs showed that one cycle of tremelimumab significantly decreased peripheral clonality, while no additional effects were seen after locoregional therapy.

Based on positive results from this and other studies evaluating immune checkpoint inhibitors in HCC,[63,64] a number of studies have been initiated to test the combination of immune checkpoint blockade (and other types of immunotherapy) plus locoregional therapies (Table 2). However, many questions in this field remain unanswered, related to mechanisms, sequencing, timing, optimal locoregional therapy and optimal immunomodulation or combination of immunomodulation. The precise and distinct immunological effects of locoregional therapies are not clear. Which treatment modality is more immunogenic? What is the best timing for the immune checkpoint blockade – before, during or after locoregional therapies? What type of TACE is most immunogenic and how do we define biomarkers of response? Can these markers only be found in tumour biopsies or can we detect them in peripheral blood? What is the best delivery mode for immunotherapy and should we focus on effector cells or immune suppressor mechanisms in the microenvironment? Asking key questions may expedite and facilitate answers with major clinical implications. Unravelling this complex threaded knot will require true team science

Table 2. Ongoing studies evaluating the combination of locoregional therapies and immunotherapy.

	CLINICAL TRIAL	Number of patients	Locoregional therapy	Immunomodulator
1	NCT03592706	60	TACE	Immune killer cells
2	NCT03575806	60	TACE	Central memory T cells
3	NCT03572582	49	TACE	Nivolumab
4	NCT03397654	26	TACE	Pembrolizumab
5	NCT03383458	530	Ablation	Nivolumab
6	NCT03143270	14	DEB-TACE	Nivolumab
7	NCT02856815	78	TACE	Immuncell-LC
8	NCT03124498	55	TACE, PEIT, RFA	Autologous CIKs
9	NCT02821754	90	TACE, RFA, Cryo	Durvalumab, Tremelimumab
10	NCT02568748	20	TACE	CIKs
11	NCT02487017	60	TACE	DCs-CIKs
12	NCT02837029	35	Y90 Glass Microspheres	Nivolumab
13	NCT03380130	40	Y90 Microspheres	Nivolumab
14	NCT03033446	40	Y90	Nivolumab
15	NCT03099564	30	Y90	Pembrolizumab
16	NCT03259867	80	TATE	Nivolumab or Pembrolizumab

CIKs, cytokine-induced killer cells; DCs, dendritic cells; DEB-TACE, drug-eluting bead-TACE; PEIT, percutaneous ethanol injection therapy; RFA, radiofrequency ablation; TACE, transarterial chemoembolization; TATE, transarterial tirapazamine embolization.

and interdisciplinary partnerships and co-education. Only a combined effort between experts in tumour immunology, hepatology and interventional oncology/locoregional therapies will enable optimal therapies to be defined for patients with hepatocellular carcinoma, who until very recently had few effective options. Indeed, it is an exciting time to have coffee with an expert from another discipline.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

All authors contributed to the manuscript.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2019.01.027.

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