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REVIEW

Advances and perspectives of dendritic cell-based active immunotherapies in follicular lymphoma

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Abstract

Follicular lymphoma (FL) is a remarkably immune-responsive malignancy, which is still considered incurable. As, standard immunochemotherapy is complex, toxic and not curative, improvement in FL care is now a crucial topic in hemato-oncology. Recently, we and others have shown that dendritic cell (DC)-based therapies allow a specific immune response associated with sustained lymphoma regression in a proportion of low-tumor burden FL patients. Importantly, the rate of objective clinical response (33–50%) and of sustained remission is remarkably higher compared to similar studies in solid tumors, corroborating the assumption of the immune responsiveness of FL. Our experimental intra-tumoral strategy combined injection with rituximab and interferon-α-derived dendritic cells (IFN-DC), a novel DC population particularly efficient in biasing T-helper response toward the Th1 type and in the cross-priming of CD8 + T cells. Noteworthy, intra-tumoral injection of DC is a new therapeutic option based on the assumption that following the induction of cancer-cell immunogenic death, unloaded DC would phagocytize in vivo the tumor associated antigens and give rise to a specific immune response. This approach allows the design of easy and inexpensive schedules. On the other hand, advanced and straightforward methods to produce clinical-grade antigenic formulations are currently under development. Both unloaded DC strategies and DC-vaccines are suited for combination with radiotherapy, immune checkpoint inhibitors, immunomodulators and metronomic chemotherapy. In fact, studies in animal models have already shown impressive results, while early-phase combination trials are ongoing. Here, we summarize the recent advances and the future perspectives of DC-based therapies in the treatment of FL patients.

Keywords Follicular lymphoma · Immunotherapy · Vaccines · Dendritic cells · IFN-alpha · Combination therapy

Abbreviations

DC	Dendritic cells
FL	Follicular lymphoma
GM-CSF	Granulocyte macrophage colony-stimulating factor
ICI	Immune checkpoint inhibitor
IFN-DC	IFN-α-conditioned dendritic cells
IFN-α	Interferon alpha
IFN-γ	Interferon gamma
IL-4-DC	Interleukine-4
KLH	Keyhole limpet hemocyanin
LTB	Low tumor burden
NHL	Non-Hodgkin lymphoma
NK	Natural killer
NOD-SCID	Non-obese diabetic/severe combined immunodeficiency
PBL	Peripheral blood lymphocytes
Relapsed/Refractory	RR
TNF-α	Tumor necrosis factor alpha
Treg	Regulatory T cells
TAA	Tumor associated antigens

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Introduction

Follicular lymphoma (FL) is by far the most frequent low-grade non-Hodgkin lymphoma. The majority of affected patients (FLs) are diagnosed in advance stage, which is still considered incurable [1]. However, in the past few decades,

following advances in both treatment and supportive care, the median overall survival of FL patients improved to 18 years. About a third of newly diagnosed, advanced-stage FL are asymptomatic, with low-tumor burden, who have no reduction in overall survival when systemic treatment is deferred until lymphoma progression [1], which occurs within a median of 30 months [2]. The standard first-line treatment foresees 6–8 cycles of immunochemotherapy (anti-CD20-antibody plus chemotherapy) delivered by long intravenous infusions, followed by maintenance with an anti-CD20-antibody [3–5]. Despite thereafter about 80% of FLs obtain a long term remission [3], this approach is complex, expensive and causes an increased rate of early and late toxicities [5, 6]. Furthermore, it is not curative, as almost all patients will eventually relapse. In addition, both the poor responders to first-line immune-chemotherapy and the 20–30% of patients who transform into high-grade lymphoma will need intensive approaches [7]. Subsequent lines of therapy are generally less and less effective and increasingly toxic, leading to a poor quality of life and a reduced life expectancy [5, 8]. Therefore, improvement in FL care is now considered a crucial topic in hemato-oncology.

Recently, breakthrough clinical studies have shown that a combination therapy based on the immunomodulator lenalidomide with rituximab is equally effective to immunochemotherapy and less toxic [9]. Although the overall impact of this advance still needs to be included, the impressive activity of this chemo-free combination highlights how the synergies of this malignancy with the tumor microenvironment and the host immune system can be successfully exploited [10]. Some unique features of FL (and other low-grade lymphomas) make this chronic cancer particularly attractive for active immunotherapies. In fact, FL is a slow growing tumor, which derives from B lymphocytes that have undergone a productive VDJ rearrangements of their immunoglobulin genes and a subsequent somatic hypermutation. Normally, due to immune tolerance, germline-derived immunoglobulin peptides are not effective targets for T cells [11]. On the contrary, the mutated idiotype (Id) contains novel antigenic determinants. This represents a class of neoantigens unique for the entire malignant B-cell clone, and it was deemed to be an ideal target for therapeutic vaccination [12, 13].

Indeed, in the 1990s and early 2000, many efforts were made in order to boost host immune response against the mutated Id of FL. However, the production of patient-tailored vaccines based on B-cell clone Id for each individual is very complex, laborious and expensive. Moreover, these vaccines elicited specific immune responses, but except for the trial by Schuster et al. [12], they failed to show a clear-cut clinical benefit in a randomized setting [13]. Nonetheless, the search for effectively empowering the host immune system against FL has never stopped. Recently, a few trials based on the concept that dendritic cells (DC) are necessary mediators for eliciting an effective immune response, yielded promising clinical results.

Conventional and IFN-α dendritic cells (IFN-DC)

Dendritic cells (DC) are professional antigen-presenting cells (APC) which play a key role in the initiation of primary immune responses [14]. Since their discovery, it has been shown that DC lineage is complex and encompasses a variety of different subsets, including: (1) conventional DC (cDC), (2) plasmacytoid DC (pDC), (3) Langerhans cells and monocyte-derived DC (moDC). DC have attracted considerable attention as potential cell drugs in the formulation of therapeutic cancer vaccines. Cancer vaccination attempts have recently been made using reinfusion of defined populations of DC obtained ex vivo from peripheral blood, including the use of BDCA1 + cDC and pDC [15]. However, the scarceness of these DC subsets in the peripheral blood has so far imposed major limitations to their use in the clinical setting. Therefore, most DC-based vaccines employed moDC differentiated from circulating monocytes cultured in the presence of IL-4 and GM-CSF and/or other cytokines, because of the relative ease of retrieving monocytes from the peripheral blood.

While the optimal culture conditions for generating the most effective moDC are still a matter of debate, our group developed an easy and rapid method for generation of partially mature and highly active moDC from blood monocytes in the presence of IFN-α and GM-CSF (IFN-DC) [16, 17]. IFN-DC present several advantages over conventional DC, especially in the clinical setting. The addition of IFN-α and GM-CSF to human monocytes results in their differentiation into activated DC showing a partially mature phenotype in only 3 days of culture, thus minimizing cell manipulation and avoiding the addition of maturation factors to the culture medium. In fact, IFN-DC express very high levels of costimulatory molecules such as CD80, CD86, CD40 and HLA-DR, as well as variable levels of the maturation marker CD83 [16, 17] and are also endowed with enhanced migratory response to chemokines [18]. Despite their partially mature phenotype, IFN-DC retain the capacity to efficiently engulf proteins and apoptotic cells [17]. While antigen-loaded IFN-DC promptly acquire a phenotype of fully mature DC upon encountering PBL [19]. IFN-DC and conventional DC obtained with IL-4, share typical DC features, but also show distinct molecular and functional phenotypes. As a result of the transcriptional signature of IFN-α, IFN-DC show a more advanced maturation and features of both plasmacytoid DC and NK cells [20, 21]. They are endowed of greater RNA levels for an array of cytokines and

chemokines like MCPs, CXCL2 and CXCL-3 which enable the effective recruitment of other innate effector cells as well as a predominant Th1 cytokine production. On the molecular level, IFN-DC also show peculiar features to initiate an adaptive immune response in the lymph node, displaying a higher expression of several molecules involved in antigen processing, migration to and localization in the lymph nodes (integrin α4 and CCR7) [20, 21].

Worthy of note, IFN-DC present a higher efficiency in targeting antigens onto class I molecules, with respect to the mature IL-4-DC counterparts [22]. In fact, IFN-DC are competent in preserving internalized proteins from early degradation, thus efficiently routing antigens toward the MHC-I processing pathway and allowing long-lasting cross-priming capacity [23]. This suggests that antigens might be efficiently retained by IFN-DC in lymphoid organs for extended periods after uptake, favoring the recruitment of rare specific CD8 + T cell precursors and increasing the probability of their interaction with the APC. Furthermore, a different pattern of proteasome activity is apparently present in IFN-DC, as IFN-α appears to boost epitope cross-presentation by DC, enhancing the expression of immunoproteasome subunits [24].

An ideal cancer vaccine should be able to trigger potent Th1-driven and CTL antitumor responses and, at the same time, to inhibit regulatory T cells (Treg). IFN-DC are seen as promising candidates for cancer immunotherapy on the basis of the handy and safe methodology for their preparation, their capacity to release a unique array of cytokines and chemokines known to efficiently favor Th1-type responses and their enhanced capacity to stimulate CD8 + T cell immunity. Priming of naïve CD4 T cells with autologous IFN-DC results in a prominent expansion of CXCR3 + IFN-γ-producing CD4 Th1 cells [25]. They are also endowed with a special attitude to produce IL-12 family cytokines [22], to express higher levels of membrane-bound IL-15 as compared to IL-4-DC [26] and were also demonstrated as directly licensed for efficient CD4-independent CD8 + cell priming [22]. Finally, we recently demonstrated that IFN-DC loaded with apoptotic lymphoma cells from FL patients and cultured for two weeks with autologous lymphocytes led to NK cell activation, a massive IFN-γ production, Th1 response skewing and enhanced cytotoxic effector function toward autologous lymphoma cells, along with a reduced capacity to induce Treg expansion [26].

Dendritic cell-based immunotherapies in follicular lymphoma: current status

DC have a pivotal role in priming specific immune responses against cancer cells. The feasibility of large-scale ex vivo generation of DC, easily obtained at one time point from patient's peripheral blood monocytes and cryopreserved in ready-for-use aliquots, allows for their clinical exploitation in cancer therapy. Of note, while a defective differentiation and functional alteration of the endogenous DC has been observed in cancer patients [27], the injection/infusion of autologous antigen-pulsed or unloaded DC, generated ex vivo, may circumvent tumor-induced dysfunction and restore immune surveillance. The following trials, through different approaches, aimed at exploiting DC-based vaccination strategies in FL (Table 1).

In 2002, Timmerman and co-workers reported on a series of 35 FL patients, treated with a personalized vaccine based on autologous Id-pulsed DC. They showed that a specific immune response was elicited in 65% of patients [28]. This was associated with objective and durable clinical responses in about a third of subjects who were relapsed/refractory (RR) after induction chemotherapy. DC were obtained by leukapheresis following density-gradient separation and loaded in vitro with tumor-derived Id protein [28]. The Id-pulsed DC-vaccine was administered by four repeated IV infusions. Noteworthy, anti-Id T cell response was shown in 62%, while an Ab response was detected in 14% of FL. Interestingly, re-vaccination using the Id-KLH conjugate to pulse DC, allowed regression, even of large tumor burdens, in patients who failed a clinical response to the initial DC-vaccine pulsed only with the Id. In addition, only the DC-Id-KLH vaccination allowed a specific IgG response. These antibodies were shown to mediate signal transduction in tumor cells by cross-linking surface immunoglobulin on B-lymphoma cells, which is known to be an initial step in the cascade of events leading to apoptosis. This approach, despite proving clinically effective and based on a strong rationale was not further pursued, as the requirements of producing a custom-made protein for each patient and limitation of the antitumor response to a single antigen were considered significant drawbacks. Conversely, DC-vaccines using whole tumor cells were shown to be more easily generated and capable of eliciting immunity against the entire collection of antigens expressed by the tumor, following their processing and presentation as exogenous cell-derived antigenic peptides, thereby evoking T cell antitumor response.

In 2009, Di Nicola and co-workers reported that 6/18 (33%) heavily pre-treated relapsed/refractory (RR) patients with FL and lymphoplasmacytic lymphoma achieved a long-term clinical remission following vaccination with four repeated doses of a DC-vaccine based on whole tumor cells [29], pre-treated with heat-shock, UV and γ-radiation in order to induce an immunogenic cell death. Worth noting, an additional 6/18 (33%) subjects remained in stable disease after vaccination at a median follow-up time of 50 months. In responders, it was observed an activation of natural killer (NK) cells, which correlated with a reduced frequency of Treg cells. Furthermore, a humoral response directed

Table 1 Major clinical trials of DC-based vaccination of FL patients: features and interpretation of results

DC-based schedule	Cycles & DC-number	Diagnosis/status	ORR (CR = n;PR = n)	FUP mo	PFS* mo median (range)	Immunological responses	Refererences
Id-pulsed or Id-KLH-pulsed DC	4 IV infusion of $2–32 \times 10^6$	Stage III–IV FL CR & RR after chemo	36% (CR = 6; PR = 4)	64	48 (1–79)	Specific T cell 62% Anti-Id Abs 14% Overall 65%	[28]
Apoptotic cell-loaded TNF-α-matured DC	4 sc. injections of $45 \pm 3 \times 10^6$ DC	Stage I–IV FL or LPL RR after chemo	33% (CR = 3; PR = 3)	50.5	45 (7–49)	Reduction in Tregs % Increase of NK % Specific T cell response Anti-HPS Abs	[29]
Unloaded immature DC, preconditioning with low-dose radiotherapy and intra-tumoral Rituximab	4 intra-tumor injections	Stage I–IV FL Untreated & Relapsed	38% (CR = 1; PR = 4)	60	14 (8–54)	Specific CD8 + & CD4 T cell	[34]
Unloaded IFN-DC preconditioning with low-dose intra-tumoral Rituximab	8 intra-tumor injections of 1×10^7 DC	Stage III–IV FL RR after chemo	50% (CR = 3; PR = 1)	26	13.5 (4–28)	Specific CD8 + & CD4 T cell	[19]

FL: follicular lymphoma; LPL: lymphoplasmacytic lymphoma; FUP: follow-up in months; CR: complete remission; PR: partial remission; PFS: progression free survival in patients who attained PR or CR

against common lymphoma antigens was demonstrated [30]. Remarkably, the extent of cell membrane translocation of the immune-stimulatory molecules calreticulin (CRT) and heat-shock protein 90 (HSP90) upon the death-inducing treatment of lymphoma cells varied in different patients and appeared to positively correlate with the clinical response to the DC-vaccine [31].

Pre-clinical studies had shown that rituximab as well as other targeted antibodies can induce the apoptosis of lymphoma cells and their uptake by DC via the Fc receptor [32, 33]. Furthermore, it was demonstrated that when DC are allowed to internalize antibody-coated lymphoma cells, the cross-presentation of tumor antigens to T cells leads to a wider and more potent immune response not primarily directed toward lymphoma Id, as compared to the uptake of untreated apoptotic cells or lysate [33].

In a following clinical study by Kolstad and co-workers [34], the potential benefit of both mAb and radiotherapy in inducing immunogenic lymphoma-cell death and thus promoting their in-vivo uploading by DC were exploited. A series of mostly untreated LTB-FL with superficial lymphoma lesions were enrolled. The schedule consisted in the combination of (1) single nodal low-dose radiotherapy and (2) intra-tumoral injection of low-dose rituximab, immature DC and GM-CSF. Upon the completion of the scheduled three cycles, five out of 14 patients (36%) had a systemic response, which was long lasting in 2/5 (40%). This abscopal effect was associated with the demonstration of a specific T cell response in the peripheral blood of 50% of all subjects. Interestingly, both specific CD8 + and at a lesser extent CD4 + specific T cell clones were shown to be elicited upon this treatment. In 2019, we published a study in RR FL, where unloaded IFN-DC were inoculated in the affected lymph nodes [19]. This was preceded 24 h before, by the intra-tumoral injection of low-dose rituximab. The schedule foresaw eight repeated cycles and resulted in an impressive ORR of 50% in eight treated subjects. Noteworthy, three out of four respondents achieved complete remission (CR) and the clinical response was long lasting in 2 CR and 1 PR patients, respectively [19] (Table 1). The finding that all patients treated with this combination therapy showed induction of tumor-specific T cell responses and the observation of the abscopal effect supports the original assumption and rationale of our study: the intranodal injection of rituximab and IFN-DC can result in an endogenous antitumor vaccination. Worth noting, all these studies were carried out in an outpatient setting, proved safe, simple to administer and very well tolerated.

Finally, we would like to report on a recent study which aimed at inducing anti-lymphoma vaccination through an in situ strategy which combined the irradiation of tumor cells to allow their immunogenic death with the recruitment and activation of DC within the targeted lymphoma lesion [35]. Following FLT3 and a TLR3 agonist injection into the irradiated lymphoma nodes, it was shown that the recruited DC could efficiently phagocyte the apoptotic cells, eliciting

a systemic T cell response. The ORR of 27% seems just slightly inferior to the previously reported studies based on the administration of DC preventively collected from the patient. Nonetheless, this research could clearly demonstrate that the cross-priming of CD8 + T cells by the TAA-loaded DC is a pivotal step, in order to elicit a clinically effective anti-lymphoma immune response.

Although it is difficult to draw definitive conclusions from these few heterogeneous early-phase trials some preliminary annotations can be made: (1) the ORR to DC-based therapies was > 30% (range 33–50%), this figure is significantly higher than in solid cancers (range 0–16%); (2) both DC-vaccine and intra-tumoral unloaded DC associated with rituximab without radiotherapy elicited immune responses as well as systemic tumor regression (3) the outbreak of autoimmune phenomenon was not an issue.

IFN-α and IFN-DC in follicular lymphoma: a new chapter of an old story

IFN-α was originally characterized for its antiviral activity [36]. However, it has been extensively used for the treatment of many types of tumors and proved the most useful and wide-ranging biologic antitumor agent in NHL as well as in other hematological cancers [37, 38].

A striking direct evidence of IFN-α activity in both B and T cell low-grade lymphomas is the regression of cutaneous and conjunctival neoplastic lesions following repeated in situ injections of this pleiotropic drug [39, 40]. This phenomenon, in the light of more recent knowledge, may be considered as proof of evidence that IFN-α, injected locally, can lead to the disruption of the tumor microenvironment and immune stimulation [41]. Furthermore, IFN-α without ribavirin was shown to allow the sustained remission of HCV-related low-grade lymphoma, by both counteracting the infection and restoring the immune functions [42], while a pivotal randomized trial in FL showed that the combination of IFN-α with standard chemotherapy significantly prolonged progression-free survival [43–45]. However, in the late 90 s, with the advent of the monoclonal anti-CD20-antibody rituximab, the use and development of IFN-α combined therapies rapidly faded down in NHL. These was also due to the relevant side effects associated with its long-term use [44]. Nonetheless, the clinical efficacy of IFN-α, in hematological malignancies and in low-grade lymphomas has been clearly demonstrated [46]**.** Noteworthy, recent knowledge on the interactions between type I IFN and DC emphasizes the importance of these cytokines in linking innate and adaptive immunity [47, 48], supporting the notion that the therapeutic effects of IFN-α in the treatment of lymphomas and leukemias could conceivably be associated to an efficient stimulation/activation of DC and NK cells [47, 48]. Accordingly, IFN-α may be involved in the in vivo conversion of circulating monocytes into powerful DC mediating the immune surveillance of tumors. Of note, naturally occurring IFN-DC has been detected in regressing molluscum contagiosum skin lesions [49] characterized by the accumulation of plasmacytoid DC (pDC) and the local production of type I IFN, suggesting a potential contribution of IFN-DC in the local immune response. Hence, it can be speculated that the exposure of monocytes to type I IFN may represent an early mechanism driving DC differentiation and activation in response to virus infection and possibly to tumors. In fact, high amounts of IFN are physiologically produced at the site of infection/inflammation acting as a danger signal. This may enable a cascade starting from the differentiation of circulating monocytes into DC, leading to the activation of natural killer cells, the generation of a Th1-polarized T-helper response and the induction of CD8 T cell response against both pathogens and cancer cells. In fact, we previously showed in vitro that IFN-DC are effectively endowed with all these functions and, under certain conditions, can even kill leukemic and lymphoma cells [16, 19, 21]. Interestingly, when IFN-α is combined with GM-CSF, it can be used to allow the differentiation of monocytes from leukemic patients into dendritic-like cells promoting anti-leukemic cytotoxicity [50–52]. Noteworthy, IFN-α was also reported to have the potential to induce a graft-versus-leukemia effect (GVL) when administered with donor leukocyte infusion (DLI) in patients who relapsed after allogeneic transplantation [53].

Studies to evaluate the direct effect of IFN-α as immune adjuvant on experimental and conventional vaccines in humans and mice have been made [54]. However, only few pilot clinical studies have attempted to evaluate the possible immune modulating activity of these cytokines in vaccination strategies [54]. In some of them, IFN-α induces improved immunological responses or enhanced peptide immunogenicity [54]. Of note, Le Bon and colleagues [55] demonstrated that DC were the cell type mediating the adjuvant effect of type I IFN in vivo, inducing long-term antibody production and immunological memory against a poorly immunogenic antigen.

For all the above, it is reasonable to hypothesized that the in vitro culture conditions devised for the generation of IFN-DCs may mimic the in vivo process which leads to the differentiation of effective APC from peripheral monocyte. This may suggest a particular suitability of IFN-DC for the design of cancer vaccine.

IFN-dendritic cells in the active immunotherapy of follicular lymphoma

We have explored two basic concepts for the development of novel IFN-DC-based therapies. Besides the standard administration of IFN-DC loaded with autologous lymphoma cells,

a second approach was perused. This relied on the concept of intra-tumoral vaccination, elicited by the sequential intranodal injection of low dose monoclonal antibodies targeted to lymphoma antigens (i.e., rituximab), followed by unloaded IFN-DC. Both approaches would have finally promoted the cross-presentation to the CD8 + T cells of tumor-associated antigens (Figs. 1 and 2).

IFN-DC loaded with an immunogenic tumor cell lysate were recently shown to elicit lymphoma-specific CTLs in both mantle cell lymphoma (MCL) and diffuse large B-cell lymphoma (DLBCL) models in vitro [56]. In addition, this IFN-DC-vaccine inhibited lymphoma growth in hu-PBL-NOD/SCID mice [56]. The analysis of T cell subpopulations showed that the antitumor immune responses were mediated by both Th1 and Th17 cells, while the high titers of IFN-γ detected in the sera of treated mice was consistent with the ability IFN-DC to induce a systemic Th1-skewed immune response [56].

We previously reported that IFN-DC generated from FL patients, loaded with apoptotic FL cells and cultured with autologous lymphocytes can induce and/or revitalize CD8 and NK cell effector functions toward autologous primary FL cells in vitro, thus promoting a robust anti-lymphoma response. We also shown IFN-DC as poor inducers of Treg-expansion and suppressive functions [26]. Worth noting, CD8 + T cell response toward FL cells was preceded by IFN-DC mediated NK-cell activation. This was associated to the enhanced expression of natural cytotoxicity receptors together with CD16 and an early sustained production of IFN-γ [30]. This cytokine is known to potently suppress the conversion of CD4 + lymphocytes into Treg cells [57]. Treg and soluble factors such as IL-10 and TGF-β seem to act together to create a tolerant environment [58]. Conversely, IFN-α can abrogate the tolerance mediated by human tolerogenic DC [59]. In fact, IL-10 and TGF-β were barely detectable in all examined cultures of PBL from FL patients cultured with autologous apoptotic tumor cell-loaded IFN-DC [26]. These observations were in accordance with other studies showing reduced Treg frequencies in PBL cultures from renal carcinoma (RC) patients stimulated with autologous IFN-DC [60] and in the peripheral blood of patients with medullary thyroid carcinoma (MTC) vaccinated with tumor lysate-pulsed IFN-DC [61].

In order to translate the in vitro results into a clinically relevant setting, IFN-DC were also evaluated in xenograft murine models of lymphoma growth [19, 62]. Interestingly, we showed that therapeutic vaccination of hu-PBL-NOD/SCID mice bearing established human lymphoma with lymphoma cell-loaded IFN-DC resulted in a significant inhibition of tumor cell growth and spread. We also found that the administration of unloaded IFN-DC induced a decrease in the microvasculature density in tumor tissues, thus suggesting that stand-alone IFN-DC can inhibit angiogenic processes or mediators [62].

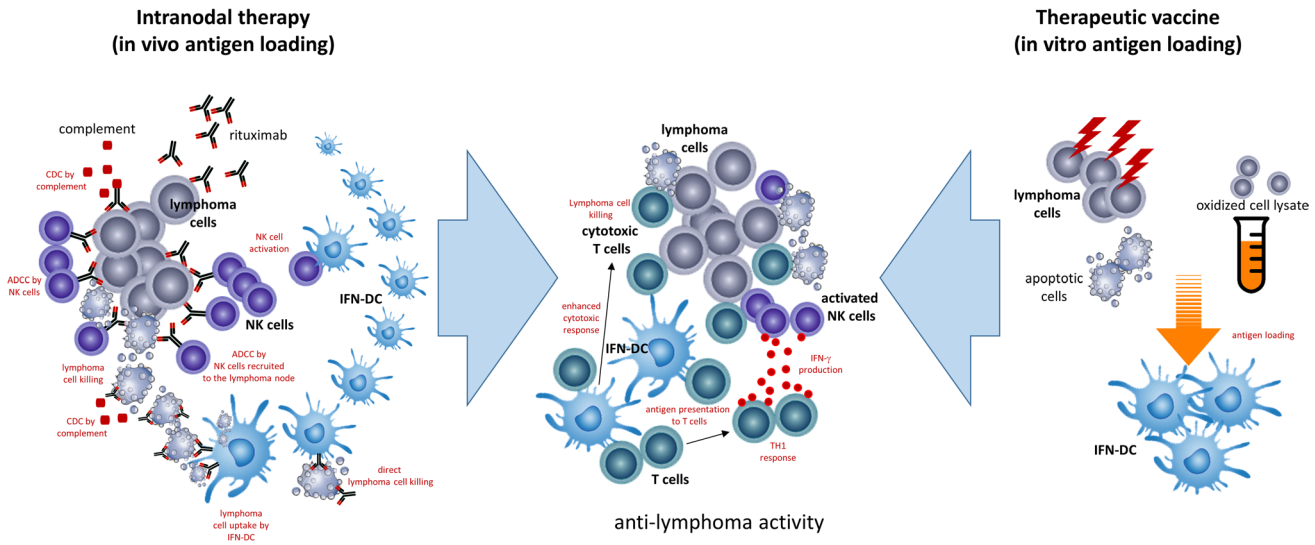

Fig. 1 Proposed mechanisms of action of IFN-DC-based therapies in follicular lymphoma**.** Intranodal therapy is based on the sequential injection of rituximab and unloaded IFN-DC into the affected lymph node (left side), which results in the enhancement of tumor apoptosis through synergistic mechanisms. Rituximab induces complement and NK-mediated antibody cytotoxicity (CDC and ADCC) of CD20-expressing FL cells and mediates FcR binding and uptake by IFN-DC. IFN-DC enhance NK cell activation and supposedly kill lymphoma cells directly. The local uptake of lymphoma-associated antigens by IFN-DC promote the presentation of tumor antigens from lymphoma cells to specific CD4 and CD8 T cells, resulting in endogenous vaccination and the migration of the T cells to distant lymphoma nodes. Vaccine formulations based on the use of a highly immunogenic tumor cell preparations loaded onto IFN-DC are suitable for all FL patients, especially those without affected superficial targetable nodes (right side). IFN-DC are preventively loaded with autologous lymphoma cells in vitro before their injection in FL patients as a personalized vaccine to induce anti-lymphoma response

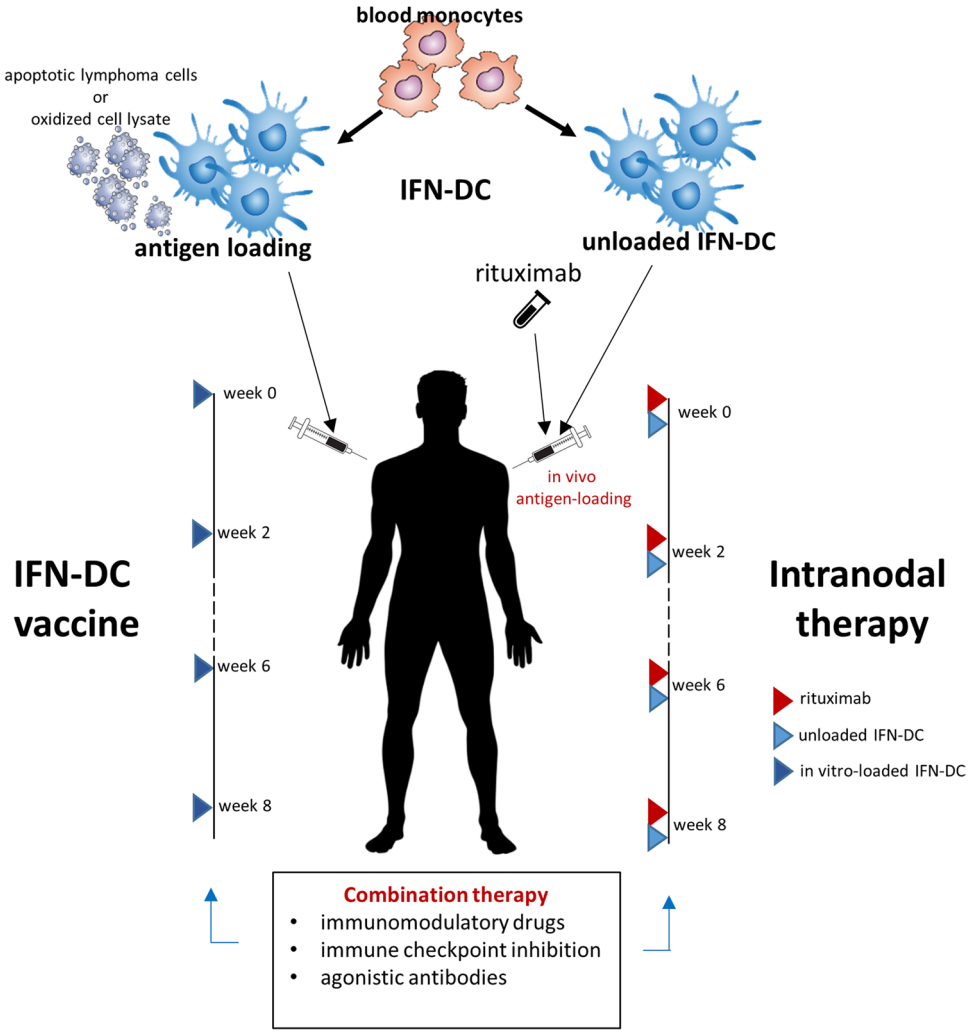

Fig. 2 Possible schedules for the clinical exploitation of IFN-DC in the treatment of follicular lymphoma**.** Enrolled patients undergo leukapheresis to collect PBMC and purify blood monocytes necessary for IFN-DC generation. Large numbers of partially-mature IFN-DC can be easily obtained at one time point from purified peripheral blood monocytes cultured in the presence IFN-α and GM-CSF, loaded or not with tumor antigens, and cryopreserved in ready-for-use aliquots for the programmed cycles of treatment. On the right is depicted the prototypical schedule of intranodal therapy already performed in the phase I trial in 8 patients with refractory and relapsed FL. It is mainly based on sequential *in situ* injections of low-dose of rituximab and unloaded IFN-DC. Rituximab administration is followed, 24 h later, by the injection of unloaded IFN-DC. The main criteria for eligibility include biopsy-confirmed indolent CD20 + follicular lymphoma, low tumor burden and superficial accessible lesions. The intranodal injections are guided by ultrasound and performed by a radiologist to ensure correct administration. The treatment cycle is repeated at two-week intervals, possibly targeting a different accessible lymphoma lesion. According to the therapeutic vaccination strategy shown in the left side of the figure, IFN are loaded in vitro with autologous lymphoma antigens and are administered intradermally, in close vicinity to axillary and inguinal lymph nodes or directly administered into a healthy lymph node. The vaccination cycles are repeated at two-week intervals. Conceivably, IFN-DC-based monotherapy can evolve in chemotherapy-free combinatorial therapy regimens with immune checkpoint inhibiting antibodies, agonistic antibodies or immunomodulating drugs

A xenograft murine model was also used to assess the concept of endogenous vaccination by the direct injection of rituximab and unloaded IFN-DC into lymphoma nodes. This schedule, in hu-PBL-NOD/SCID mice, resulted in the infiltration of lymphoma xenografts by human CD8 lymphocytes thus providing the rationale for the phase I clinical trial [19]. This study (Table 1) provided the first evidence on the safety and clinical activity of unloaded IFN-DC and rituximab used for the endogenous vaccination of FL patients [19]. The mechanisms of endogenous vaccination were designed to act through repeated waves of rituximab-induced lymphoma cell killing, followed by the Fc-receptor-mediated phagocytosis of tumor antigens by IFN-DC (Figs. 1 and 2). Indeed, the regression of lymphoma lesions distal to the injection site was considered proof of concept that this mechanism of

endogenous immunization was elicited in vivo. Tumor-specific responses directed toward clonal IGVH-predicted epitopes were detected in the peripheral blood of the 4 patients exhibiting a partial or complete clinical response, showing a peak at 6–9 months after the beginning of the treatment. Worth noting, lymphocyte cytolytic responses were not only directed toward class-I-restricted CD8 idiotype epitopes but also to class-II-restricted CD4 epitopes. This was not unexpected, as lymphomas are B-cell tumors expressing on their surface both MHC class I and II alleles and short peptides derived from Ig V-regions are almost exclusively displayed on MHC class II molecules. In fact, it was shown that the idiotype neoantigens arising by somatic hypermutation events, unique to each B-cell can be recognized by naturally occurring cognate cytotoxic CD4 T cells. These have been identified and can mediate the killing of autologous lymphoma cells [11, 63, 64]. Although in our trial, no significant correlations were found between clinical response and tumor-specific immune response, it must be emphasized that either CD4 or CD8 T cell responses toward clonal IGVH epitopes were observed in clinically responding patients. Even though a wider T cell response to specific and shared unknown FL-associated (mutated) antigens was supposedly elicited by intranodal vaccination, this could not be monitored in the course of the trial, owing to the lack of a suitable read-out system. This study represented the first proof of principle on the activity of intra-tumoral unloaded IFN-DCs combined with Rituximab in inducing tumor regression, which occurred in 50% of treated subjects. While the induction of tumor-specific T cells was observed in all of them, thus inferring the occurrence of a successful endogenous anticancer vaccination. Noteworthy, regression of untreated lesions was observed in all respondents, thus suggesting the occurrence of a systemic response. The rationale and treatment schedule of our study was somehow similar to that followed by Kolstad and colleagues [34]. However, most of the subjects enrolled in his study were mostly treatment-naïve as compared to those enrolled in our study who were heavily pretreated. Furthermore, there are some significant differences in our study that deserve to be emphasized: (i) the omission of radiotherapy, which can be an additional burden for patients, considering that rituximab alone may be sufficient to promote the release of tumor antigens; (ii) the use of IFN-DCs compared to the immature IL-4-DC; (iii) a lower number of DCs used for each treatment cycle (1×10^7 cells/cycle vs. $5–10 \times 10^7$); (iv) the higher number of treatment cycles (8 vs. 3), which could be essential to elicit a long-lasting anticancer response. Although the limited number of patients evaluated in our study does not allow to draw any conclusion on clinical efficacy, the antitumor response observed in some patients with multiple relapsed or refractory FL, was quite impressive.

Alternative approaches for developing DC-based vaccines

Recently, it was demonstrated that whole autologous lymphoma cells can represent an optimal antigenic formulation for DC loading [26, 29]. Compared to the anti-Id vaccination, this strategy is less labor intensive and more affordable. Above all, it offers the advantage of presenting multiple tumor antigens contained within FL cells, thus enabling a wider and more efficient antitumor immune response. Therefore, the development new GMP-grade vaccine formulations based on the use of a highly immunogenic tumor cell preparations loaded onto DC and suitable for FL patients without affected superficial nodes would represent a major advance in the field. As we previously published, IFN-DC loaded with lymphoma cells induced to undergo immunogenic cell death are powerful inducers of specific anti-lymphoma immune response. Autologous lymphoma cells can be easily purified from lymph node biopsies and used as an antigen source to load patient-derived IFN-DC. Nonetheless, GMP-compliant standards impose major limitations to the clinical translation of autologous follicular lymphoma apoptotic cells as an antigen source. Microbiological testing, including sterility testing for bacterial and fungal contamination must be performed on cell preparations, in-process intermediates and the final product in order to ensure its safety. Notably, lymph nodes may harbor living bacteria, especially in elderly or immunocompromised patients. Lymph nodes may also be contaminated by bacteria during its resection or handling. In both cases, the final product fails to meet the release criteria for its clinical use. Consequently, the development of safe and efficient FL antigen formulations remains a pressing need for the clinical exploitation of tumor cell-loaded IFN-DC for therapeutic vaccines. We are currently exploring the use whole tumor lysate preparation with hypochlorous acid (HOCl) oxidation to induce rapid necrosis and increase the immunogenicity of tumor cells. HOCl is a strong bactericidal capable of potentiating the immunogenicity of proteins [65] and potentially applicable to many cancer types, including lymphomas. Interestingly, in recent years, lymphoma cell-derived extracellular vesicle (LCEV) have raised much interest for their therapeutic potential, including the development of cancer vaccines [66]. LCEV are demonstrated to carry tumor derived molecules, mRNAs as well as membrane antigens, including CD20, and are actively released in blood as well as biological fluids. Although there is still a lack of efficient technology platforms for their isolation, LCEV can be selectively isolated from the circulation and

potentially used to load DC as alternative antigen sources [66].

Combination therapies with IFN-DC

Despite DC intranodal therapy has proved effective in generating detectable T cell responses against lymphoma id determinants, it is likely that the full potential of DC-therapies in lymphoma will only be realized when these are combined with other drugs targeting tumor immunosuppressive mechanisms and/or boosting the ongoing immune response, thus increasing the activity of immune effector cells. In fact, several mechanism, such as (1) the development of tumor-associated macrophages, (2) the induction of regulatory Tregs; (3) the accumulation of myeloid-derived suppressor cells (MDSCs), and (4) the expression of inhibitory checkpoints such as PD-1 on CD8 + T cells, may all contribute to impair immune effector functions and vaccinal efficacy. Therefore, the next logical step is to evaluate in humans both unloaded DC and DC-vaccine schedules combined with clinically approved immunomodulatory drugs or immune checkpoint inhibitors (ICI), which are known to improve the T cell response or to diminish restraints at different levels. These combinations already proved effective in different experimental models [67–69] and are currently being tested in ongoing clinical trials. Also agonistic antibodies, enhancing T cell costimulation [70] should act synergistically with IFN-DC-based therapies to augment anti-lymphoma immunity.

Immunotherapies based on ICI have convincingly led to impressive clinical responses [71, 72], thus rekindling the enthusiasm toward immunotherapy and tumor antigen vaccination. Only in the light of these recent successes with ICI, therapeutic vaccination for cancer patients has been seriously reconsidered. Thus, IFN-DC-based immunotherapy and T cell checkpoint modulation can act as synergic partners, since checkpoint inhibitors are merely immune drivers of pre-existing immune responses and their efficacy is proportional to the extent of pre-existing tumor-specific T cell. In this view, for its best efficacy, ICI should rely on preventive priming and expansion of tumor-specific T cells by active immunization. This also makes biological sense, since tumors with low mutational burden as FL may evoke a weak antitumor immunity, a defect that active DC-based vaccination typically aims to correct. Notably, in our recent study, an increase in pre-existing T cell responses against the mutated Id of FL patients and de novo response was observed after intranodal therapy with IFN-DC [19]. Moreover, new findings suggest that full activation of T cells by the ICI anti-PD-1 is not direct, but rather involves tumor-infiltrating DC producing IL-12 upon interaction with neighboring T cells releasing IFN-γ [73].

A particularly attractive drug to be combined with IFN-DC-based therapies is lenalidomide, as it acts through both the boosting of antitumor immunity and the modification of tumor microenvironment.[74]. Previous studies demonstrated that a combination of DC-vaccination and lenalidomide can efficiently enhance antitumor immune response in murine models of multiple myeloma, via the inhibition of immunosuppressive cells and the enhancement of CD8 cell responses [69]. We have recently combined this pleiotropic drug with an IFN-DC-based lymphoma vaccine [62]. Lenalidomide was shown to enhance anti-lymphoma cytotoxicity of human PBL stimulated with IFN-DC in vitro and to promote a remarkable reduction in Treg frequency, thus demonstrating a boosting effect of anti-lymphoma effector cell functions [62]. The treatment of xenochimeric mice bearing established lymphoma with either IFN-DC vaccination or lenalidomide led to a significant decrease in tumor growth and lymphoma cell spread. However, only the combined treatment led to the massive regression of established lymphomas [62]. A strong rationale would also support the triple combination of intra-tumoral rituximab and IFN-DC plus systemic lenalidomide. In fact, lenalidomide has been shown to synergize with rituximab by enhancing NK-mediated ADCC and lymphoma cell killing through complementary mechanisms [10]. This synergy should increase antigen availability and uptake by IFN-DC, thus improving the cross-presentation of lymphoma antigens to CD8 + cells. However, it should be mentioned that a phase I trial in chronic lymphocytic leukemia (CLL) based on a DC-vaccine combined with lenalidomide was prematurely closed because of the outbreak of autoimmune cytopenias [75]. Nevertheless, as autoimmune phenomena are frequently observed in CLL patients, we believe this preliminary experience should not prevent further trials of this combination in other clinical settings.

The chance to integrate a new IFN-DC-vaccines in future combinatorial immunotherapy regimens with ICI, agonistic antibodies or immunomodulating drugs hold hopes and expectations for the management of FL.

Perspectives and future directions

Importantly, the few early-phase DC-based trials carried out in FL have all shown a remarkable rate of objective clinical response and sustained remission as compared to similar studies in solid tumors [12, 19, 29, 34]. These results, while confirming FL as an exquisitely immune-responsive cancer, allow to hypothesize that DC-based immunotherapies may become an integral part of advanced treatments for low-grade B-cell lymphomas. However, to translate these promising results into a clinical perspective, it is necessary to both empower DC-based schedules and to define

their applicability in specific clinical settings. Combination agents, useful for improving the activity of DC should ideally (1) reduce the tumor mass, as high disease burdens harness the clinical efficacy of DC-schedules; (2) not impair the immune system of the host; (3) release the brakes of ongoing immune response; (4) synergize directly or indirectly with DC functions. Several agents as radiotherapy, monoclonal antibodies, immunomodulators, standard and metronomic chemotherapies, are endowed with these features and may allow effective combinations. Indeed, in patients with LTB indolent lymphomas, the true challenge would be to counteract the disease before it evolves to a more aggressive and incurable condition. At this purpose, also unloaded DC may be conveniently used to devise easy and inexpensive protocols. A number of studies already confirmed the activity of low-dose radiotherapy to induce immunogenic cell death, which is a sine-qua-non condition for the suitability of unloaded DC. In addition, ongoing studies are already exploring the combination with checkpoint blocking antibodies and pro-inflammatory stimuli.

Our previous clinical trial showed that intranodal injection of unloaded IFN-DC is not only safe and feasible, but also exhibits promising clinical efficacy in FL patients. However, the applicability of schedules employing in vivo DC-loading is restricted to patients who have accessible superficial tumor lesions. Undeniably, this limitation must be overcome. Thus, new methodologies for the straightforward generation of clinical-grade tumor cell-derived antigen formulations for DC loading may open new perspectives for their wide application in FL, especially as a consolidation strategy. On the other hand, it was already shown both in animals and humans that the injection of in vitro-generated DC into the lymphatic system allows their effective migration to functionally active lymph nodes (LN). Therefore, unloaded DC may migrate through the lymphatic vessels and upload the tumor antigens derived from lymphoma cell death induced by systemic anti-cancer treatments, in draining lymph nodes.

The advent of the new chemo-free combination treatment based on lenalidomide and anti-CD20 [10] promise to be a suitable milieu for the combination with active immunotherapies [6]. Conversely standard immunochemotherapy which results in a long-standing immune-suppression would not allow to envisage an effective synergy [76]. Lastly as anti-CD20-based maintenance allows a prolonged progression-free survival (PFS) but not a survival advantage in FL [3], maintenance may become another scenario to be challenged by novel DC-based therapies.

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Compliance with ethical standards

Conflict of interest Stefano M. Santini received research funding from Celgene. All other authors declare that they have no conflict of interest.

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References

1. Freedman A (2018) Follicular lymphoma: 2018 update on diagnosis and management. Am J Hematol 93:296–305. https://doi.org/10.1002/ajh.24937
2. Ardeshna KM, Qian W, Smith P et al (2014) Rituximab versus a watch-and-wait approach in patients with advanced-stage, asymptomatic, non-bulky follicular lymphoma: an open-label randomised phase 3 trial. Lancet Oncol 15:424–435. https://doi.org/10.1016/S1470-2045(14)70027-0
3. Bachy E, Seymour JF, Feugier P et al (2019) Sustained progression-free survival benefit of rituximab maintenance in patients with follicular lymphoma: long-term results of the prima study. J Clin Oncol 37:2815–2824. https://doi.org/10.1200/JCO.19.01073
4. Marcus R, Davies A, Ando K et al (2017) Obinutuzumab for the first-line treatment of follicular lymphoma. N Engl J Med 377:1331–1344. https://doi.org/10.1056/NEJMoa1614598
5. Casulo C, Nastoupil L, Fowler NH et al (2017) Unmet needs in the first-line treatment of follicular lymphoma. Ann Oncol Off J Eur Soc Med Oncol 28:2094–2106. https://doi.org/10.1093/annonc/mdx189
6. Hiddemann W, Barbui AM, Canales MA et al (2018) Immunochemotherapy with obinutuzumab or rituximab for previously untreated follicular lymphoma in the gallium study: influence of chemotherapy on efficacy and safety. J Clin Oncol 36:2395–2404. https://doi.org/10.1200/JCO.2017.76.8960
7. Freeman CL, Kridel R, Moccia AA et al (2019) Early progression after bendamustine-rituximab is associated with high risk of transformation in advanced stage follicular lymphoma. Blood 134:761–764. https://doi.org/10.1182/blood.2019000258
8. Maddocks K, Barr PM, Cheson BD et al (2017) Recommendations for clinical trial development in follicular lymphoma. J Natl Cancer Inst. https://doi.org/10.1093/jnci/djw255
9. Morschhauser F, Fowler NH, Feugier P et al (2018) Rituximab plus lenalidomide in advanced untreated follicular lymphoma. N Engl J Med 379:934–947. https://doi.org/10.1056/NEJMoa1805104
10. Chiu H, Trisal P, Bjorklund C et al (2019) Combination lenalidomide-rituximab immunotherapy activates anti-tumour immunity and induces tumour cell death by complementary mechanisms of action in follicular lymphoma. Br J Haematol 185:240–253. https://doi.org/10.1111/bjh.15797
11. Bogen B, Ruffini P (2009) Review: to what extent are T cells tolerant to immunoglobulin variable regions? Scand J Immunol 70:526–530. https://doi.org/10.1111/j.1365-3083.2009.02340.x
12. Schuster SJ, Neelapu SS, Gause BL et al (2011) Vaccination with patient-specific tumor-derived antigen in first remission

improves disease-free survival in follicular lymphoma. J Clin Oncol 29:2787–2794. https://doi.org/10.1200/JCO.2010.33.3005
13. Levy R, Ganjoo KN, Leonard JP et al (2014) Active idiotypic vaccination versus control immunotherapy for follicular lymphoma. J Clin Oncol 32:1797–1803. https://doi.org/10.1200/JCO.2012.43.9273
14. Wculek SK, Cueto FJ, Mujal AM et al (2019) Dendritic cells in cancer immunology and immunotherapy. Nat Rev Immunol. https://doi.org/10.1038/s41577-019-0210-z
15. Wimmers F, Schreibelt G, Sköld AE et al (2014) Paradigm shift in dendritic cell-based immunotherapy: from in vitro generated monocyte-derived DCs to naturally circulating DC subsets. Front Immunol 5:165. https://doi.org/10.3389/fimmu.2014.00165
16. Santini SM, Lapenta C, Logozzi M et al (2000) Type I interferon as a powerful adjuvant for monocyte-derived dendritic cell development and activity in vitro and in Hu-PBL-SCID mice. J Exp Med 191:1777–1788. https://doi.org/10.1084/jem.191.10.1777
17. Santini SM, Lapenta C, Santodonato L et al (2009) IFN-alpha in the generation of dendritic cells for cancer immunotherapy. Handb Exp Pharmacol. https://doi.org/10.1007/978-3-540-71029-5_14
18. Parlato S, Santini SM, Lapenta C et al (2001) Expression of CCR-7, MIP-3beta, and Th-1 chemokines in type I IFN-induced monocyte-derived dendritic cells: importance for the rapid acquisition of potent migratory and functional activities. Blood 98:3022–3029. https://doi.org/10.1182/blood.v98.10.3022
19. Cox MC, Castiello L, Mattei M et al (2019) Clinical and antitumor immune responses in relapsed/refractory follicular lymphoma patients after intranodal injections of IFNα-dendritic cells and rituximab: a phase I clinical trial. Clin Cancer Res 25:5231–5241. https://doi.org/10.1158/1078-0432.CCR-19-0709
20. Stroncek DF, Basil C, Nagorsen D et al (2005) Delayed polarization of mononuclear phagocyte transcriptional program by type I interferon isoforms. J Transl Med 3:24. https://doi.org/10.1186/1479-5876-3-24
21. Korthals M, Safaian N, Kronenwett R et al (2007) Monocyte derived dendritic cells generated by IFN-alpha acquire mature dendritic and natural killer cell properties as shown by gene expression analysis. J Transl Med 5:46. https://doi.org/10.1186/1479-5876-5-46
22. Lapenta C, Santini SM, Spada M et al (2006) IFN-alpha-conditioned dendritic cells are highly efficient in inducing cross-priming CD8(+) T cells against exogenous viral antigens. Eur J Immunol 36:2046–2060. https://doi.org/10.1002/eji.200535579
23. Spadaro F, Lapenta C, Donati S et al (2012) IFN-α enhances cross-presentation in human dendritic cells by modulating antigen survival, endocytic routing, and processing. Blood 119:1407–1417. https://doi.org/10.1182/blood-2011-06-363564
24. Lattanzi L, Rozera C, Marescotti D et al (2011) IFN-α boosts epitope cross-presentation by dendritic cells via modulation of proteasome activity. Immunobiology 216:537–547. https://doi.org/10.1016/j.imbio.2010.10.003
25. Santini SM, Lapenta C, Donati S et al (2011) Interferon-α-conditioned human monocytes combine a Th1-orienting attitude with the induction of autologous Th17 responses: role of IL-23 and IL-12. PLoS ONE 6:e17364. https://doi.org/10.1371/journal.pone.0017364
26. Lapenta C, Donati S, Spadaro F et al (2016) NK cell activation in the antitumor response induced by IFN-α dendritic cells loaded with apoptotic cells from follicular lymphoma patients. J Immunol 197:795–806. https://doi.org/10.4049/jimmunol.1600262
27. Bandola-Simon J, Roche PA (2019) Dysfunction of antigen processing and presentation by dendritic cells in cancer. Mol Immunol 113:31–37. https://doi.org/10.1016/j.molimm.2018.03.025
28. Timmerman JM, Czerwinski DK, Davis TA et al (2002) Idiotype-pulsed dendritic cell vaccination for B-cell lymphoma: clinical and immune responses in 35 patients. Blood 99:1517–1526. https://doi.org/10.1182/blood.V99.5.1517
29. Di Nicola M, Zappasodi R, Carlo-Stella C et al (2009) Vaccination with autologous tumor-loaded dendritic cells induces clinical and immunologic responses in indolent B-cell lymphoma patients with relapsed and measurable disease: a pilot study. Blood 113:18–27. https://doi.org/10.1182/blood-2008-06-165654
30. Zappasodi R, Bongarzone I, Ghedini GC et al (2011) Serological identification of HSP105 as a novel non-Hodgkin lymphoma therapeutic target. Blood 118:4421–4430. https://doi.org/10.1182/blood-2011-06-364570
31. Zappasodi R, Pupa SM, Ghedini GC et al (2010) Improved clinical outcome in indolent B-cell lymphoma patients vaccinated with autologous tumor cells experiencing immunogenic death. Cancer Res 70:9062–9072. https://doi.org/10.1158/0008-5472.CAN-10-1825
32. Selenko N, Majdic O, Draxler S et al (2001) CD20 antibody (C2B8)-induced apoptosis of lymphoma cells promotes phagocytosis by dendritic cells and cross-priming of CD8+ cytotoxic T cells. Leukemia 15:1619–1626. https://doi.org/10.1038/sj.leu.2402226
33. Franki SN, Steward KK, Betting DJ et al (2008) Dendritic cells loaded with apoptotic antibody-coated tumor cells provide protective immunity against B-cell lymphoma in vivo. Blood 111:1504–1511. https://doi.org/10.1182/blood-2007-03-080507
34. Kolstad A, Kumari S, Walczak M et al (2015) Sequential intranodal immunotherapy induces antitumor immunity and correlated regression of disseminated follicular lymphoma. Blood 125:82–89. https://doi.org/10.1182/blood-2014-07-592162
35. Hammerich L, Marron TU, Upadhyay R et al (2019) Systemic clinical tumor regressions and potentiation of PD1 blockade with in situ vaccination. Nat Med 25:814–824. https://doi.org/10.1038/s41591-019-0410-x
36. Vilcek J (2006) Fifty years of interferon research: aiming at a moving target. Immunity 25:343–348. https://doi.org/10.1016/j.immuni.2006.08.008
37. Guilhot F, Roy L, Guilhot J, Millot F (2004) Interferon therapy in chronic myelogenous leukemia. Hematol Oncol Clin North Am 18:585–603. https://doi.org/10.1016/j.hoc.2004.03.002
38. Morroni M, Cinti S (1995) Hairy cell leukemia: an ultrastructural study of hairy cells before and after interferon therapy. Tumori 81:249–55
39. Zinzani PL, Magagnoli M, Galieni P et al (1999) Nongastrointestinal low-grade mucosa-associated lymphoid tissue lymphoma: analysis of 75 patients. J Clin Oncol 17:1254. https://doi.org/10.1200/JCO.1999.17.4.1254
40. Blasi MA, Tiberti AC, Valente P et al (2012) Intralesional interferon-α for conjunctival mucosa-associated lymphoid tissue lymphoma: long-term results. Ophthalmology 119:494–500. https://doi.org/10.1016/j.ophtha.2011.09.008
41. Aricò E, Castiello L, Capone I et al (2019) Type i interferons and cancer: An evolving story demanding novel clinical applications. Cancers (Basel). https://doi.org/10.3390/cancers11121943
42. Hermine O, Lefrère F, Bronowicki J-P et al (2002) Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. N Engl J Med 347:89–94. https://doi.org/10.1056/NEJMoa013376
43. Cannata-Ortiz J, Nicolás C, García-Noblejas A et al (2019) Rituximab, interferon-alfa-2b and dose dense CVP is highly efficient in patients with FLIPI ≥ 2 follicular lymphoma. Final results of the LNH-PRO-05 study. Br J Haematol 186:168–170. https://doi.org/10.1111/bjh.15760
44. Cole BF, Solal-Céligny P, Gelber RD et al (1998) Quality-of-life-adjusted survival analysis of interferon alfa-2b treatment for advanced follicular lymphoma: an aid to clinical decision

making. J Clin Oncol 16:2339–2344. https://doi.org/10.1200/JCO.1998.16.7.2339
45. Arranz R, García-Alfonso P, Sobrino P et al (1998) Role of interferon alfa-2b in the induction and maintenance treatment of low-grade non-Hodgkin's lymphoma: results from a prospective, multicenter trial with double randomization. J Clin Oncol 16:1538–1546. https://doi.org/10.1200/JCO.1998.16.4.1538
46. Rohatiner AZS, Gregory WM, Peterson B et al (2005) Meta-analysis to evaluate the role of interferon in follicular lymphoma. J Clin Oncol 23:2215–2223. https://doi.org/10.1200/JCO.2005.06.146
47. Schiavoni G, Mattei F, Gabriele L (2013) Type I interferons as stimulators of DC-mediated cross-priming: impact on anti-tumor response. Front Immunol 4:483. https://doi.org/10.3389/fimmu.2013.00483
48. Gessani S, Conti L, Del Cornò M, Belardelli F (2014) Type I interferons as regulators of human antigen presenting cell functions. Toxins (Basel) 6:1696–1723. https://doi.org/10.3390/toxins6061696
49. Vermi W, Fisogni S, Salogni L et al (2011) Spontaneous regression of highly immunogenic Molluscum contagiosum virus (MCV)-induced skin lesions is associated with plasmacytoid dendritic cells and IFN-DC infiltration. J Invest Dermatol 131:426–434. https://doi.org/10.1038/jid.2010.256
50. Hirn Lopez A, Deen D, Fischer Z et al (2019) Role of interferon (IFN)α in "Cocktails" for the generation of (Leukemia-derived) dendritic cells (DCleu) from blasts in blood from patients (pts) with acute myeloid leukemia (AML) and the Induction of anti-leukemic reactions. J Immunother 42:143–161. https://doi.org/10.1097/CJI.0000000000000266
51. Gabriele L, Borghi P, Rozera C et al (2004) IFN-alpha promotes the rapid differentiation of monocytes from patients with chronic myeloid leukemia into activated dendritic cells tuned to undergo full maturation after LPS treatment. Blood 103:980–987. https://doi.org/10.1182/blood-2003-03-0981
52. Bialek-Waldmann JK, Heuser M, Ganser A, Stripecke R (2019) Monocytes reprogrammed with lentiviral vectors co-expressing GM-CSF, IFN-α2 and antigens for personalized immune therapy of acute leukemia pre- or post-stem cell transplantation. Cancer Immunol Immunother 68:1891–1899. https://doi.org/10.1007/s00262-019-02406-9
53. Grigg A, Kannan K, Schwarer AP et al (2001) Chemotherapy and granulocyte colony stimulating factor-mobilized blood cell infusion followed by interferon-alpha for relapsed malignancy after allogeneic bone marrow transplantation. Intern Med J 31:15–22. https://doi.org/10.1046/j.1445-5994.2001.00013.x
54. Rizza P, Moretti F, Capone I, Belardelli F (2015) Role of type I interferon in inducing a protective immune response: perspectives for clinical applications. Cytokine Growth Factor Rev 26:195–201. https://doi.org/10.1016/j.cytogfr.2014.10.002
55. Le Bon A, Schiavoni G, D'Agostino G et al (2001) Type I interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo. Immunity 14:461–470. https://doi.org/10.1016/S1074-7613(01)00126-1
56. Montico B, Lapenta C, Ravo M et al (2017) Exploiting a new strategy to induce immunogenic cell death to improve dendritic cell-based vaccines for lymphoma immunotherapy. Oncoimmunology 6:e1356964. https://doi.org/10.1080/2162402X.2017.1356964
57. Brillard E, Pallandre J-R, Chalmers D et al (2007) Natural killer cells prevent CD28-mediated Foxp3 transcription in CD4+CD25- T lymphocytes. Exp Hematol 35:416–425. https://doi.org/10.1016/j.exphem.2006.12.004
58. Pedroza-Pacheco I, Madrigal A, Saudemont A (2013) Interaction between natural killer cells and regulatory T cells: perspectives for immunotherapy. Cell Mol Immunol 10:222–229. https://doi.org/10.1038/cmi.2013.2
59. Bacher N, Graulich E, Jonuleit H et al (2011) Interferon-α abrogates tolerance induction by human tolerogenic dendritic cells. PLoS ONE 6:e22763. https://doi.org/10.1371/journal.pone.0022763
60. Gigante M, Mandic M, Wesa AK et al (2008) Interferon-alpha (IFN-alpha)-conditioned DC preferentially stimulate type-1 and limit Treg-type in vitro T-cell responses from RCC patients. J Immunother 31:254–262. https://doi.org/10.1097/CJI.0b013e318167b023
61. Papewalis C, Wuttke M, Jacobs B et al (2008) Dendritic cell vaccination induces tumor epitope-specific Th1 immune response in medullary thyroid carcinoma. Horm Metab Res 40:108–116. https://doi.org/10.1055/s-2007-1022565
62. Lapenta C, Donati S, Spadaro F et al (2019) Lenalidomide improves the therapeutic effect of an interferon-α-dendritic cell-based lymphoma vaccine. Cancer Immunol Immunother. https://doi.org/10.1007/s00262-019-02411-y
63. Weng J, Baio FE, Moriarty KE et al (2016) Targeting B-cell malignancies through human B-cell receptor specific CD4 $^{+}$ T cells. Oncoimmunology 5:e1232220. https://doi.org/10.1080/2162402X.2016.1232220
64. Khodadoust MS, Olsson N, Chen B et al (2019) B-cell lymphomas present immunoglobulin neoantigens. Blood 133:878–881. https://doi.org/10.1182/blood-2018-06-845156
65. Chiang CL-L, Kandalaft LE, Tanyi J et al (2013) A dendritic cell vaccine pulsed with autologous hypochlorous acid-oxidized ovarian cancer lysate primes effective broad antitumor immunity: from bench to bedside. Clin Cancer Res 19:4801–4815. https://doi.org/10.1158/1078-0432.CCR-13-1185
66. Liu J, Wang X (2019) Focus on exosomes—From pathogenic mechanisms to the potential clinical application value in lymphoma. J Cell Biochem 120:19220–19228. https://doi.org/10.1002/jcb.29241
67. Vo M-C, Anh-NguyenThi T, Lee H-J et al (2017) Lenalidomide enhances the function of dendritic cells generated from patients with multiple myeloma. Exp Hematol 46:48–55. https://doi.org/10.1016/j.exphem.2016.11.004
68. Sakamaki I, Kwak LW, Cha SC et al (2014) Lenalidomide enhances the protective effect of a therapeutic vaccine and reverses immune suppression in mice bearing established lymphomas. Leukemia 28:329–337. https://doi.org/10.1038/leu.2013.177
69. Nguyen-Pham T-N, Jung S-H, Vo M-C et al (2015) Lenalidomide synergistically enhances the effect of dendritic cell vaccination in a model of murine multiple myeloma. J Immunother 38:330–339. https://doi.org/10.1097/CJI.0000000000000097
70. Chester C, Sanmamed MF, Wang J, Melero I (2018) Immunotherapy targeting 4–1BB: mechanistic rationale, clinical results, and future strategies. Blood 131:49–57. https://doi.org/10.1182/blood-2017-06-741041
71. Ansell SM, Lesokhin AM, Borrello I et al (2015) PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med 372:311–319. https://doi.org/10.1056/NEJMoa1411087
72. Westin JR, Chu F, Zhang M et al (2014) Safety and activity of PD1 blockade by pidilizumab in combination with rituximab in patients with relapsed follicular lymphoma: a single group, open-label, phase 2 trial. Lancet Oncol 15:69–77. https://doi.org/10.1016/S1470-2045(13)70551-5
73. Garris CS, Arlauckas SP, Kohler RH et al (2018) Successful Anti-PD-1 cancer immunotherapy requires T cell-dendritic cell crosstalk involving the cytokines IFN-γ and IL-12. Immunity 49:1148–1161.e7. https://doi.org/10.1016/j.immuni.2018.09.024
74. Witzig TE, Nowakowski GS, Habermann TM et al (2015) A comprehensive review of lenalidomide therapy for B-cell non-Hodgkin lymphoma. Ann Oncol Off J Eur Soc Med Oncol 26:1667–1677. https://doi.org/10.1093/annonc/mdv102

75. Palma M, Hansson L, Mulder TA et al (2018) Lenalidomide as immune adjuvant to a dendritic cell vaccine in chronic lymphocytic leukemia patients. Eur J Haematol 101:68–77. https://doi.org/10.1111/ejh.13065
76. Cox MC, Battella S, La Scaleia R et al (2015) Tumor-associated and immunochemotherapy-dependent long-term alterations of the peripheral blood NK cell compartment in DLBCL patients. Oncoimmunology 4:1–12. https://doi.org/10.4161/21624 02X.2014.990773