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Review

Towards Possible Cure of Cancer by Immunotherapy of Minimal Residual Disease Using Intentionally Mismatched Donor Lymphocytes

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Abstract

Immunotherapy is now recognized as the most promising approach for control of cancer cells resistant to available anti-cancer modalities, yet, cure in patients with advanced and resistant disease is nearly never accomplished. Preliminary data summarized in the present mini-review provides support to a new treatment strategy for treatment of patients with hematological malignancies and solid tumors, based on a 2-step approach: first, an attempt to apply a stage of minimal residual disease (MRD) by conventional modalities; next, using optimal immunotherapy preferably at an early stage of the disease during MRD focusing on intentionally mismatched, Alloreactive Targeted Activated Cancer Killer cells (ATACK) following well-tolerated immunosuppressive conditioning. Preliminary clinical investigations confirm that short-term circulation of ATACK, including T cells and natural killer (NK) cells activated with IL-2 prior to and following cell infusion can kill by reverse rejection-like mechanism allogeneic malignant target cells, cancer cells and cancer stem cells alike, resulting in cure. Our working hypothesis supported by pre-clinical and preliminary clinical investigations confirming the feasibility to cure cancer by ATACK, justify prospective randomized clinical trials to investigate the feasibility and efficacy of ATACK against cancer.

Keywords: immunotherapy of cancer, intentionally mismatched donor lymphocytes, immunotherapy of hematological malignancies, immunotherapy of solid tumors, Cell-mediate immunotherapy; allogeneic donor lymphocytes, IL-2 activated killer cells; cure of cancer

Introduction

Cancer continues to be a leading cause of death worldwide. Considering lack of information about the causes of malignant transformation, except when viral agents seem to be involved, the universal dogma for treatment of cancer is based on 'the more the better' using available anti-cancer agents, attempting to eradicate the primary tumor by optimal resection, followed by chemotherapy or radiation therapy when indicated, considering additional treatment only as soon as secondary metastases become visible, usually focusing on aggressive chemotherapy as the standard of care. For patients with cancer not expected to respond adequately to conventional treatment, especially for treatment of hematological malignancies, myeloablative chemo-radiotherapy followed by rescue with stem cell transplantation may be considered in an attempt to eradicate otherwise resistant malignant cells. Over the years it became apparent that in patients with primary resistant cancer or with metastatic disease, cure is unlikely to be accomplished. Indeed, relapse continues to be the single major obstacle in treatment of hematologic malignancies and many solid tumors as well, even following initial complete remission [1,2]. On the other hand, as the intensity of the regimen used for treatment of cancer or using high dose chemotherapy supported by stem cell transplantation (SCT) is escalated, the risks of procedure-related toxicity and mortality increase and recurrent disease may not be preventable [3]. In addition, increased incidence of late complications in patients treated with repeated courses of chemotherapy that may or may not increase survival are likely to impair the quality of life of long-term survivors and their supporting family. It became apparent that newer modalities must be introduced in order to improve the cure rate of patients with hematological malignancies and solid tumors as well as to improve the quality of life of successfully treated patients. For patients resistant to available chemotherapy, immunotherapy became in recent years an acceptable treatment as it was confirmed that activating patient's immune system could be effective even against otherwise resistant disease. Unfortunately, in the absence of tumor-specific antigens and the unresponsiveness that develops between the immune system and the rapidly developing cancer cells, immunotherapy based on an attempt to immunize patients against cancer or using anti-cancer vaccines is far from being effective [4]. Clearly, the goal of effective immunotherapy should be based on an attempt to break the existing tolerance of the immune system against cancer cells, or

in other words to induce an 'autoimmune-like' response against tumor cells recognized as 'self' by patient's unresponsive/tolerant immune system [5]. To date, effective tumor-associated antibodies, such as anti-CD20 in patients with B cell non-Hodgkin's lymphoma as well as additional monoclonal antibodies against other cancer-associated antigens (e.g. Her2/nu; EGFR; VEGF to mention just a few) may be effective by induction of antibody-dependent cell-mediated cytotoxicity for control tumor progression in responding patients but these are usually not sufficient for complete eradication of the disease [6].

Considering the fact that cancer cells mutate spontaneously as well as consistently acquire resistance to chemotherapy and other available anti-cancer modalities, especially considering the fact that cancer stem cells are a priori resistant to available anti-cancer agents, new approaches are urgently indicated for treatment of otherwise incurable cancer. At present, despite major progress, and innovative use of checkpoint inhibitors, with few exceptions, cancer remains incurable disease. Therefore, newer strategies are needed in an attempt to control malignant cells resistant to chemotherapy.

As will be suggested in this short article, cell-mediated immunotherapy by alloreactive lymphocytes that can be targeted against cancer cells at the stage of minimal residual disease (MRD) which can be consistently accomplished in a large majority of patients at an early stage of the disease may represent one of the most promising approaches for cure of cancer. Aiming for cure, application of any effective anti-cancer modality focusing on immunotherapy provides the optimal, sometimes the only, opportunity for cure.

Considering the fact that there are no two cancers that are exactly the same and no two patients that are exactly the same, treatment of cancer should be fully personalized, both cancer-specific and patient-specific. As will be documented herewith, cell-mediated immunotherapy by alloreactive, preferably haploidentical, donor lymphocytes may represent one of the most promising approaches to accomplish cure when applied at the stage of minimal residual disease, certainly in patients with hematological malignancies.

The Role of Immunotherapy by Alloreactive Lymphocytes

Following successful animal experiments, the clinical role of allogeneic lymphocytes was introduced in patients with leukemia successfully treated by allogeneic SCT. The role of allogeneic SCT, explored originally by Thomas and colleagues in the early 1970s became the treatment of choice for/ patients resistant to conventional doses of chemotherapy, and subsequently for patients at high-risk to relapse following maximally tolerated doses of conventional chemotherapy [7]. Subsequently, SCT was successfully utilized for the treatment of genetic diseases and other life-threatening non-malignant indications using the same therapeutic principles for replacement of abnormal host hematopoietic cells with donor hematopoietic cells.

Traditionally, it was considered that high-dose chemoradiotherapy was the main component in the bone marrow transplant procedure and that transplantation of genotypically or phenotypically matched stem cells was mainly indicated for rescue of the lethally treated recipient. Hence, much attention was given to maximize tumor cell kill by maximally tolerated doses of chemotherapy (single agents and combinations of non-cross-reactive agents).

However, it was recognized for many years that the incidence of relapse was high among recipients rescued with autologous or even syngeneic grafts as compared with recipients of allogeneic stem cells. The documented correlation between one of the most serious complications of allogeneic SCT, graft-versus-host disease (GVHD) and successful eradication of all malignant cells, suggest that immune-mediated graft-versus-leukemia (GVL) effects played a major role in elimination of residual tumor cells escaping chemoradiotherapy [8-11].

The possibility that allogeneic lymphocytes administered in the course of SCT eliminate leukemia through immune-mediated GVL effects has been suggested ever since the earliest days of experimental [12-19] and clinical SCT [8-11]. Convincing direct correlation between acute and chronic GVHD and reduced rate of relapse of leukemia in clinical practice was first reported by Weiden et al. [8,9].

Similarly, in analogy to GVL effects, graft-versus-tumor (GVT) effects were also described in a murine model of spontaneous sarcoma [20] and more recently in metastatic breast cancer as well [21,22], as well as in preliminary trials in man [23-25]. The role of immune-mediated GVL effects in the course of SCT was further supported by observations suggesting that relapse while patients were on immunosuppressive treatment with preliminary trials in man [23-25].

The role of immune-mediated GVL effects in the course of SCT was further supported by observations suggesting that relapse while patients were on immunosuppressive treatment with cyclosporine A (CSA) was occasionally reversed by discontinuing immunosuppression [26]. Likewise, it has been documented that the incidence of relapse is lower in patients treated with sub-optimal doses of CSA used as prophylaxis against GVHD [27]. Similarly, data in mice inoculated with murine leukemia treated by SCT indicated that GVL effects mediated by mismatched donor's bone marrow cells were totally abrogated by concomitant administration of CSA for 10 days [28].

All of the above suggest that allogeneic SCT provided immunocompetent allogeneic donor T lymphocytes, which could react against residual tumor cells of host origin. Hence, the advantage of SCT over conventional chemotherapy lies in the combined effects of the myeloablative dose of chemoradiotherapy given pre-transplantation and the ability of immunocompetent allogeneic donor T lymphocytes to eliminate residual tumor cells of host origin, giving rise to GVL and GVT effects or in fact graft versus any undesirable hematopoietic cells of host origin, including genetically abnormal stem cells or their progeny [29-32].

Interestingly, similarly to the data first reported in mice [14-18], GVL effects occasionally independently of GVHD were also confirmed in clinical practice either following SCT [11] or following donor lymphocyte infusion (DLI) administered post transplantation to induce GVL effects to treat or prevent relapse when patients are off any post-transplant immunosuppressive agents, as will be detailed below [33-40].

Based on pre-clinical animal models the feasibility of induction of post-transplant GVL or GVT effects induced by alloreactive donor lymphocytes, including both T cells and NK cells present in the allografts, we hypothesized that cell-therapy

with donor lymphocytes given post grafting, especially in patients with no spontaneous GVHD following discontinuation of post-transplant anti-GVHD prophylaxis, may induce effective anti-tumor responses [5].

The Use of Durable Engraftment of Alloreactive Donor Lymphocytes Following Allogeneic SCT for Immunotherapy of Cancer

Based on the beneficial role of allogeneic stem cell transplantation in comparison with autologous stem cell transplantation and especially transplantation of patients following equal high dose chemotherapy supported by stem cell transplantation obtained from identical twins, we hypothesized that allogeneic lymphocytes of donor origin can be given post grafting for treatment as well as for prevention of relapse in high-risk cases. Indeed, the first successful case where GVL effects were induced by alloreactive DLI in a patient with resistant acute lymphoblastic leukemia (ALL) fully resistant to supra-lethal chemo-radiotherapy, followed by hundreds of patients successfully treated in Israel, subsequently supported by the cumulative international experience in a variety of malignant hematologic diseases that confirmed unequivocally the therapeutic potential of alloreactive lymphocytes [3-40].

The first patient successfully treated by DLI for relapse following SCT was a 30-month-old boy that was referred for SCT at the Hadassah University Hospital /in Jerusalem in November 1986 [33-35]. He had been diagnosed as pre-B ALL and relapsed on therapy twice. In December 1986, allogeneic SCT was carried out from a fully matched sister during second resistant relapse. Supra-lethal conditioning included total body irradiation (TBI) 1,200 cGy (two daily fractions of 200 cGy on days -6, -5 and -4) followed by two doses of cyclophosphamide 60 mg/kg (days -3 and -2) and melphalan 60 mg/m2 (day -1). The patient showed no signs of acute GVHD. At one-month post-SCT, the patient presented with full hematologic relapse and several bulky masses confirmed as extramedullary disease, including a progressing

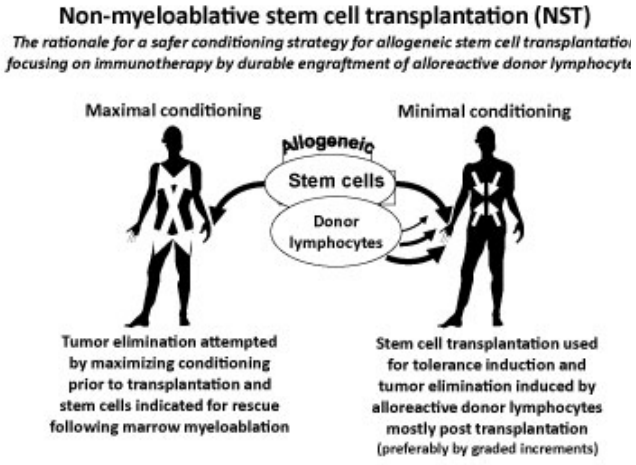

Figure 1. Non-myeloablative stem cell transplantation (NST). The rationale for a safer conditioning strategy for allogeneic stem cell transplantation focusing on immunotherapy by durable engraftment of alloreactive donor lymphocytes. A. The conventional myeloablative stem cell transplantation procedure; B. Reduced intensity conditioning or NST, focusing on immunotherapy of cancer by durable engraftment of alloreactive donor lymphocytes accomplished by engraftment of donor hematopoietic stem cells. Graded increments of donor lymphocytes post grafting can maximize GVL or GVT effects.

retro-tracheal mass necessitating an emergency tracheotomy. He responded to increments of donor (sister) peripheral blood lymphocytes infusions to induce GVL effects. The patient developed grade II GVHD with involvement of the skin and liver. He responded to a short course of corticosteroids and within 2 weeks the palpable masses decreased in size, peripheral blood and bone marrow morphology normalized and cytogenetic analysis confirmed 100% normal female karyotype in all 50 metaphases investigated. To date, more than 30 years following treatment with DLI patient is with no evidence of disease and no residual male cells can be detected by cytogenetic analysis or PCR. The efficacy of DLI for eradication of malignant cells fully resistant to lethal doses of chemo-radiotherapy was confirmed by many centers (36-40) and currently, DLI is considered the treatment of choice for patients relapsing following allogeneic stem cell transplantation [41].

Considering the anti-cancer potential of alloreactive donor lymphocytes, the use of pre-emptive DLI was introduced by our team for prevention of relapse following SCT [42]. The therapeutic role of DLI could be further maximized by in vitro or in vivo activation of alloreactivity of lymphocytes by interleukin-2 [35]. In later years, the well-documented role of alloreactive donor lymphocytes lead to the replacement of myeloablative conditioning for allogeneic SCT with reduced intensity conditioning (RIC) and non-myeloablative stem cell transplantation (NST) [43,44] as documented diagrammatically in Figure 1. Subsequently, the role of reduced intensity conditioning (RIC) was pioneered for the treatment of solid tumors as well, confirming the anti-cancer effects of alloreactive donor lymphocytes also against metastatic solid tumors [45-47

The Therapeutic Role of Non-Engrafting, Transiently Circulating Intentionally Mismatched, Alloreactive Targeted Activated Cancer Killer Cells (ATACK)

Based on the aforementioned information, we assumed that the therapeutic role of allogeneic lymphocytes could be further maximized using intentionally mismatched, haploidentical or even unrelated donor lymphocytes, and even more effectively by IL-2 activation of lymphocytes, both NK and T cells, prior to and also following cell infusion for rapid and more effective elimination of malignant cells while avoiding the use of prior SCT. The working hypothesis was that effective induction of GVL or GVT could be accomplished by short circulation of non-engrafting lymphocytes following mild immunosuppressive conditioning. The idea was to compensate the short duration of intentionally mismatched lymphocytes in the circulation until rejection by using much more effective GVL- or GVT-inducing lymphocytes. The purpose of the immunosuppressive conditioning was to combine several purposes: (1) reduction of the number and suppression of host lymphocytes to mildly extend the circulation time of donor lymphocytes; (2) establishing a "niche" for newly infused donor-derived killer cells; (3) providing an optimal infra-structure for homeostatic proliferation of alloreactive donor as well as uncommitted newly derived host lymphocytes; (4) suppression of regulatory T cells and other host-derived suppressor cells.

The successful use of intentionally mismatched donor lymphocytes, alloactivated prior to cell infusion and following cell

infusion by IL-2 using ATACK, was pioneered in a 12 years old patient with AML resistant to myeloablative chemotherapy even following autologous stem cell transplantation in 1992 [48]. Although a stage of MRD was induced by prior autologous stem cell transplantation, residual disease was still documented. Luckily, MRD was successfully eliminated by infusion of haploidentically mismatched maternal lymphocytes activated with IL-2 in vitro for 4 days prior to cell infusion and subsequently following cell infusion too by subcutaneous administration of low dose IL-2 for 5 consecutive days to allow continuous activation of alloreactive donor lymphocytes until their anticipated mandatory rejection [48]. Obviously, consistent rejection of intentionally mismatched lymphocytes prevented any risk of GVHD. Currently, 28 years later, the young girl, now a qualified MD with 2 children, is alive and well with no further treatment, free of any late complications or any signs of chronic GVHD. This patient was the first one to confirm that even transient circulation of alloreactive donor lymphocytes can eliminate malignant cells fully resistant to maximally tolerated doses of myeloablative chemotherapy. Using ATACK following well-tolerated immunosuppressive conditioning, with no prior SCT, anticipated graft-vs-tumor (GVT) effects can be maximized, while preventing any risk of GVHD, simply because mismatched lymphocytes are always rejected spontaneously. It should be noted that intentional engraftment of fully mismatched hematopoietic stem cells is extremely hard to accomplish even following myeloablative conditioning, since rejection of fully matched and even haploidentical hematopoietic stem cells can occur even following maximally tolerated myeloablative conditioning. Rejection of purified haploidentical stem cells, T cell depleted or CD34-enriched, is even harder to be accomplished even following myeloablative conditioning, thus ensuring that durable engraftment of haploidentical or mismatched unrelated lymphocytes that could cause GVHD is unlikely with no prior induction of transplantation tolerance, as was already confirmed by ongoing application of ATACK. Since the successful outcome of our first patient, using ATACK for eradication of chemotherapy resistant disease was also documented in patients with multiple myeloma and non-Hodgkin's lymphoma, with indications suggesting that similar effects may be also accomplished in patients with metastatic solid tumors, especially if ATACK could be applied at the stage of MRD [41, 45, 49, 50]. Our cumulative experience, which needs to be extended in prospective randomized clinical trials, suggests that at the stage of MRD, cure of patients with hematologic malignancies and possibly also metastatic solid tumors otherwise considered incurable, may benefit or even cured by ATACK [50].

Immunotherapy of Cancer by Selective Targeting ATACK Against Cancer Associated Antigens by Monoclonal or Bispecific Antibodies

The selectivity and efficacy of ATACK against cancer can be further improved by targeting intentionally mismatched activated killer cells against malignant cells by monoclonal or bispecific antibodies [51]. Moreover, treatment of minimal residual disease with antibody targeted killer cells may also result in induction of long-lasting anti-cancer immunity, most likely because the Fc portion of the monoclonal or bispecific antibody can bind to antigen presenting cells (dendritic cells or macrophages) leading to processing of cancer antigens and presentation to helper T cells [51, 52].

Immunotherapy of cancer with monoclonal or bispecific antibodies may be an effective way to induce antibody-dependent cell-mediated cytotoxicity which can be accomplished by using commercially available antibodies such as anti-CD20 (Rituximab), anti Her-2/neu (Herceptin), anti-EGFR (Erbitux), anti-VEGF (Avastin) and bispecific antibodies such as anti-CD3 x anti-EpCAM (Catumaxomab) that were already applied in clinical practice.

Whereas the use of transient circulation of ATACK makes it possible to eliminate truly minimal residual disease. In addition, successful activation of patient's own immune system during the GVL or GVT induced by ATACK against cancer can sometimes recruit patient's own T cells to induce long-lasting immunity that can serve as vaccination by memory T cells against residual or re-emerging malignant cells. indeed, we have already documented the feasibility of cure of cancer in parallel with induction of long-lasting anti-cancer vaccination by targeting ATACK against cancer-associated epithelial cell adhesion molecules (EpCAM) in a pre-clinical animal model by a single treatment that results in elimination of existing cancer cells by alloreactive lymphocytes, followed by induction of host derived memory T cells that can resist a lethal tumor challenge long after rejection of donor lymphocytes and after elimination of the bispecific antibody (52).

Other Supportive Methods in Preparation for Optimal Treatment of Resistant Residual Disease in Patients with Cancer

Methods to stimulate patients' own immune system against cancer include non-specific activation of T cells and NK cells with IL-2 and especially by combining treatment with IL-2 and alpha interferon. The use of interferon may amplify the anti-cancer effects of IL-2 by exposing cell surface MHC and possibly additional cancer-associated antigens on the cell surface of cancer cells. in addition, alpha interferon may also Increase the number and enhance the function of NK cells activated by IL-2. Interferon enhances cytokine secretion and degranulation of NK cells and as such increases the cytotoxic potential of NK cells that are also activated by IL-2. We have previously documented that such treatment may be effective for treatment of leukemia/lymphoma in pre-clinical animal experiments [5, 53] and in pilot clinical trials [54,55]. Treatment with IL-2 and alpha interferon significantly improved the disease-free survival and overall survival of patients with Hodgkin's and non-Hodgkin's lymphoma treated with high dose chemotherapy and autologous stem cell transplantation prior to cytokine treatment [56,57]. The anti-cancer effects of interferon alpha may also be enhanced by activation of M1 macrophages [58]. In addition, activation of macrophages can also enhance their function as antigen presenting cells [59] .

There seems to be no question that additional approaches may be also be used for synergistic activation of patient's own immune system against cancer, such as Coley's toxin and oncolytic viruses may prove effective as alternative strategies for breaking the unresponsiveness that exist between patient's T cells and cancer. In parallel, induction of anti-cancer effects may

be accomplished by successful intra-tumor vaccination using an existing tumor as an internal vaccine [60] future anti-cancer vaccination using metronomic treatment against negative regulators of the immune system such as regulatory T cells [61], checkpoint inhibitors (e.g. CTLA-4 and PD-1/PDL-1) [62,63], myeloid-derived suppressor cells [64], mesenchymal stromal cells (MSC) [65]. These negative regulators can block development of anti-cancer immunity by systemic effects or at the level of the protective tumor microenvironment.

Control of cancer cells by cancer-specific low molecular weight compounds such as tyrosine kinase inhibitors for patients with Philadelphia-positive CML represent one of the truly breakthrough treatment of cancer that could be helpful to accomplish a stage of MRD or possibly cure ceratin cancers [66, 67]. Effective anti-angiogenesis may also contribute accomplishing a stage of MRD, yet unlikely to eliminate all existing malignant cells [68]. Ongoing investigations resulting in precision medicine may also eventually lead to effective procedures for selective targeting cancer cells but until then, immunotherapy seems to present treatment of choice for an increasing number of indications.

Perhaps one of the most promising strategies currently used successfully for treatment of cancer using patient's own killer cells, at present especially effective against malignant B cell disorders, B cell leukemias and non-Hodgkin lymphomas, is the CAR-T technology based on targeting patient's own activated T cells against malignant cells [69, 70]. Unfortunately, although treatment with CAR-T induces most effective remissions even in patients with multi-drug resistant hematological disorders recurrent disease cannot be consistently eliminated, thus, most centers prefer to follow treatment with CAR-T with allogeneic stem cell transplantation to control residual malignant cells. Furthermore, CAR-T treatment is frequently accompanied by hazardous cytokine release syndrome. Production of CAR-T is most expensive, time consuming, complicated and involves the use of genetic manipulation of expanded T cells obtained from the patient processed in specially designed GMP facilities. It seems reasonable to predict that future procedures based on CAR-T technology will also focus on the use of allogeneic or even off-the-shelf readily available donor T and NK cells.

Oncolytic viruses are also most promising future agents to eradicate malignant cells resistant to available anti-cancer modalities, either due to direct cytolytic effects of oncolytic viruses and/or due to induction of effective anti-cancer immunity against "modified self", cancer cells "decorating" and modifying cancer cells with viral antigens [71]. As such, the use of cancer targeting oncolytic viruses may represent a method for combining direct anti-cancer cytotoxicity with induction of anti-cancer immunotherapy by breaking T cells-cancer tolerance.

Another interesting future treatment of cancer including elimination of cancer stem cells may involve delivery of anti-cancer molecules such as tumor suppressor microRNAs or oncolytic viruses by MSCs that naturally migrate to and target cancer cells and as such can be used as messengers of anti-cancer agents [72]. As such, neutralizing the immunosuppressive effects of MSCs in the tumor microenvironment may be accomplished by loading MSCs with agents that will use such MSCs as anti-cancer chaperons.

Discussion

Immunotherapy is now recognized as one of the most promising approaches for control, possibly even for eliminate cancer cells resistant to available anti-cancer modalities, including cancer stem cells that are a prioi resistant to chemotherapy and radiation therapy. Most if not all available immunotherapy procedures are based on an attempt to activate patient's own immune system against patient's own malignant cells, either by controlling negative regulators of the immune system including regulatory T cells and using checkpoint inhibitors on the one hand, or activation of anti-cancer effector mechanisms by cytokines, using monoclonal antibodies that can induce antibody-dependent cell-mediated cytotoxicity or targeting larger number of spontaneously generated tumor-reactive T cells, tumor-infiltrating lymphocytes (TIL) or the most recent advanced targeting of patient's own T cells against cancer-associated antigens by chimeric antigen receptor T cells (CAR-T), on the other. Yet, cure of patients with advanced and resistant malignant disease is rarely accomplished.

As suggested by this paper, optimal treatment of cancer aiming for cure, should focus on two principles: first, focusing on an attempt to eliminate MRD at an early stage of the disease which can be accomplished in the large majority of patients with cancer following successful conventional treatment; second, application of optimal anti-cancer immunotherapy at the stage of MRD. As documented by pre-clinical animal experiments [5, 15, 18, 21, 22, 73] and our successful pilot clinical investigations [25, 41, 47, 48, 50], ATACK represents a safe method for elimination of fully resistant residual malignant cells.

The first approach consists of the conventional standard of care, based on fully personalized treatment that now should also include precision medicine and additional relevant procedures such as hormonal treatment, immunotherapy by monoclonal antibodies, kinase inhibitors etc., as indicated. As suggested by our current working hypothesis supported by most encouraging pilot clinical investigations, the second approach, preferably applicable at the stage of MRD against otherwise persistent malignant cells, may result in cure because of the capacity of IL-2 activated killer cells to kill any mismatched target cells including cancer cells resistant to chemotherapy including cancer stem cell. Ideally, the common practice of experts of infectious diseases that always recommend aggressive treatment of bacterial infections, should also be applied by oncologists and hematologists for treatment of cancer, as long as eradication of MRD could be accomplished by a safe immunotherapy program, such as the one recommended here with. As such, the goal should be to recognize the high-risk cases upfront and treat MRD by immunotherapy, even if residual malignant cells cannot be visible, since cancer micro-metastases of one million cells or less are never visible and invisible cancer stem cells are a priori resistant to chemotherapy and ionizing radiation. In sharp contrast, no cure can be anticipated by treatment of patients with a heavy tumor load, late in the course of the disease, when uncontrolled metastases are already visible in patients with poor performance status, after failure of multiple courses of ineffective chemotherapy.

The "take home" message seems to be that at the stage of minimal residual disease targeted anti-cancer modalities focusing on innovative immunotherapy may provide the optimal stage for successful treatment and possibly the only chance to cure the

disease. Indeed, the goal should remain to cure cancer which probably can be accomplished by smart immunotherapy such as ATACK when applied at the stage of MRD, as confirmed in pre-clinical animal models [5, 15, 18, 21, 22, 73] and by cumulative successful clinical experience [25, 41, 47, 48, 50], rather than regarding cancer as an incurable chronic disease that needs to be treated only when the patient is symptomatic or with obvious evidence of disease. Taken together, the feasibility to eradicate otherwise resistant malignant cells at the stage of MRD should serve as a reminder to consider immunotherapy at the stage of MRD even in asymptomatic patients with high-risk disease. Based on our most encouraging preliminary experience, our working hypothesis should be further investigated in prospective randomized clinical trials by combining optimal conventional anti-cancer modalities and ATACK mediated by short-term circulation of most potent non-engrafting killer cells, best starting in patients with hematological malignancies, focusing on acute leukemia and multiple myeloma.

Abbreviations

MRD: minimal residual disease: ATACK, Allogeneic Targeted Activated Cancer Killer cells : NK cells, natural killer cells: SCT: stem cell transplantation, DLI, donor lymphocyte infusion: GVHD: graft-vs-host disease: GVL: graft-versus-leukemia effects, GVT, graft-vs-tumor effects: NST: non-myeloablative stem cell transplantation: RIC: reduced intensity conditioning

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Declaration

Prof. Slavin is currently involved in treatment of patients with cancer focusing on immunotherapy in his private clinic in Tel Aviv.

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