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Editorial

The rationale behind autologous autoimmune hematopoietic stem cell transplant conditioning regimens: concerns over the use of total-body irradiation in systemic sclerosis

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Summary:

Hematopoietic stem cell transplantation (HSCT) is becoming an increasingly recognized indication for treatment of autoimmune diseases and severe immune-mediated disorders. However, multicenter registry data have demonstrated higher than anticipated early toxicity, approximately 10% for autoimmune diseases in general, and 20–27% for diffuse systemic sclerosis (scleroderma). If uncorrected, this high treatment-related mortality will hinder development of stem cell therapy for immune-mediated diseases. In order to develop safer regimens, we address some pitfalls and concepts involved in design and selection of conditioning regimens for autoimmune diseases in general, and because it is associated with the highest regimen-related toxicity, scleroderma in specific.
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Rationale for design of autologous autoimmune HSCT regimens

For patients with malignant diseases, autologous transplant regimens are based on dose escalation of agents effective at conventional nontransplant doses. Dose escalation of a noneffective or even worse a disease-exacerbating agent would increase toxicity without additional benefit. This same reasoning should, where possible, be applied to autoimmune disorders.

Unlike malignancies where visceral organ impairment is a contraindication to HSCT, disease-related organ dysfunction is often the indication for HSCT of autoimmune diseases. Implicit in using HSCT in treating end organ damage is that the conditioning regimen should avoid agents that can potentially damage the organs that the treatment is designed to salvage. For example, agents such as bleomycin, BCNU (carmustine), and radiation that are complicated by pulmonary fibrosis would not be the logical conditioning agents for a disease such as scleroderma, mixed connective tissue disease, or dermatomyositis in which a major cause of death is related to pulmonary fibrosis and pulmonary artery hypertension.[1–4]

Conditioning regimen design should also avoid agents that could damage organ-specific stem cell compartments that may contribute to organ repair after the disease remits. The effect of conditioning agents on tissue-specific stem cell viability and proliferative potential is unknown. However, even low-dose total-body irradiation (TBI) is myeloablative, that is, lethal for hematopoietic stem cells, and in animal models, cranial radiation (10 cGy) impairs mechanism of central nervous system repair.[5] Radiation-mediated neural stem cell injury may occur by mechanisms such as apoptosis, alteration in cell cycle progression, and destruction of the neural stem cell niche or milieu through invasion of macrophages and microglia.[5] It is, therefore, reasonable to assume that myeloablative agents, which are by definition cidal for marrow stem cells, may damage or be lethal for stem cells in other organ systems.

In contrast to allogeneic HSCT for autoimmune disease in which the goal would be to replace the stem cell predisposition towards disease with a foreign and presumably nondisease-prone stem cell compartment, the rationale for autologous HSCT of autoimmune diseases is to regenerate a new, that is, antigen naïve immune system,

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from the patient's own hematopoietic stem cells. Immune reconstitution will require the re-emergence of thymic educated virgin (antigen naïve) T cells. The goal of autologous stem cell conditioning regimens is to reduce or eliminate the immune reactions that contribute to the pathologic process. In contrast to conditioning regimens for hematologic malignancies, there is no need to sterilize the marrow stem cell population. Hematopoietic stem cell destruction from myeloablative agents such as TBI would be an unwanted side effect of autologous regimens in which the goal is immune ablation not myeloablation. Intense immune ablation without myeloablative side effects could be accomplished with agents such as cyclophosphamide, fludarabine, and antibodies to T cells (antithymocyte globulin) and/or B cells (rituximab) or both T and B cells (CAMPATH or alemtuzumad®).

Treatment of autoimmune diseases with radiation

While chemotherapy has replaced total nodal irradiation (TNI) as the standard of therapy for Hodgkin's disease,[6,7] the initial success of TNI in treating Hodgkin's disease was impetus for similar trials in autoimmune disorders, the results of which were, unfortunately, discouraging. These experiences tell us that success from using radiation in malignancies does not translate into similar successful outcomes for autoimmune diseases. In general, studies on small numbers of patients did not support development of radiation for either efficacy or randomized trials or acceptance as a standard of care in autoimmune diseases. With the exception of very low-dose radiation (180–200 cGy divided as 10 cGy given two to three times a week) for myasthenia gravis,[8] total-body irradiation (TBI), total lymphoid irradiation (TLI), or total nodal irradiation (TNI) has not been an effective therapy for autoimmune diseases.

Localized deep X-ray therapy was used extensively from 1935 to 1955 for ankylosing spondylitis (AS) but thereafter abandoned when reports began appearing of at least a five-fold increase in leukemia and 60% excess in deaths from solid cancers.[9–13] Also in the 1960s, as more nonsteroidal anti-inflammatory drugs (NSAID) became available, NSAID became an effective alternative to radiation for treatment for AS.

TLI (200 cGy/day, 5 days a week for 2 weeks to a total of 2000 cGy) was used as a therapy for systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) in small numbers of patients during the 1970s and 1980s.[14–16] TLI is immune suppressive and reduces antinuclear and anti-DNA antibodies in patients with SLE.[17] However, in a study from Stanford of 21 patients with lupus nephritis, overall survival, time to dialysis, and disease control without use of other immunosuppressive agents did not appear different from conventional treatment and TBI-related secondary malignancies appeared higher than anticipated.[16] Four of 21 patients (20%) developed malignancies (two lymphomas, one thyroid cancer, one cervical cancer).[16] Severe acute toxicity occurred using the Stanford TLI protocol in a similar SLE study performed in Jerusalem.[15] In two severely ill SLE patients, following TLI, one developed herpes zoster, Gram-negative septicemia, neurologic symptoms, and deterioration of lupus nephritis.[15] The other died 2 weeks after completing treatment from bronchopneumonia, necrotic skin lesions, and progressive nephritis.[15] The acute severe side effects in the Jerusalem study were significantly worse than in the Stanford study and attributed to selection of patients with active disease at the time of treatment. Therefore, TLI in SLE patients with highly active disease appears to be poorly tolerated, while in relatively stable patients it is complicated by an increase in late malignancies and appears no more effective than the standard of care available in the 1970 s and 1980 s.

TLI (200 cGy/day, 5 days a week for 2 weeks to a total of 2000 cGy) has also been used to treat RA.[14] A retrospective comparison of the 15–20 year survival of 53 TLI-treated RA patients with 106 DMARD-treated control patients again raised concerns in the TLI-treated patients. Despite TLI, patients continued to have disease defined by high mean health assessment questionnaire scores (HAQ). Overall mortality following TLI was 25/53 (47%) *vs* 45/106 (43%) for controls. While early survival appeared better with TLI, the survival curves crossed, and by 15 years TLI-treated patient survival was 25 *vs* 50% for the control group. No patient in the control group developed a malignancy, while five TLI-treated patients developed malignancies (three lymphomas and two myelodysplastic syndromes). For patients with rheumatoid arthritis (RA), TBI at a dose of 300–400 cGy in two upper and lower hemi-body settings separated several months apart was given to four patients.[18] One patient developed myelodysplasia and another developed acute myelogenous leukemia 40 and 25 months after TBI, respectively. The other two continued to need disease modifying antirheumatoid drugs (methotrexate or cyclophosphamide) following treatment. Owing to toxicity and the development of effective biologic response modifiers such as antitumor necrosis factor, radiation studies in RA have fallen out of favor.

It appears that in autoimmune diseases such as AS, SLE, and RA, both TBI and TLI are complicated by an unacceptable rate of secondary malignancies including lymphoma, leukemia, myelodysplasia, and solid tumors. Whether other immune-suppressive drugs may be additive to the mutagenic effect of radiation or the diseases themselves are associated with an increased risk of malignant transformation following DNA damage is unknown. Peripheral blood lymphocytes obtained from patients with SLE, juvenile rheumatoid arthritis, and scleroderma have been reported to have significantly slower repair of single-stranded DNA breaks following *ex vivo* irradiation with 1.5 Gy compared to healthy controls.[19] DNA repair mechanisms are an important and evolutionarily conserved mechanism of genome maintenance.[20,21] Quantity and function of DNA repair enzymes within chronically activated immune cells remain an underinvestigated area. Because radiation is generally considered contraindicated for scleroderma (discussed below), there are no data on late tumorigenic effects of TBI or TLI in patients with scleroderma.

Treatment of scleroderma with radiation

Concerns about acute toxicity from radiation therapy in scleroderma are prominently reported in the medical literature.[22–37] Scleroderma is a vascular disease marked by fibrosis, similar to what is often observed in late radiation injury. In normal individuals (ie no autoimmune disease), radiation therapy has been reported to be the initiating event for development of localized scleroderma usually within the radiation port but also at sites distal to the radiation field.[22–29]

In patients with scleroderma, numerous papers have reported severe radiation-induced toxicity.[30–34] When using high-dose (40–70 cGy) localized radiation to treat cancer in patients with scleroderma, radiation-related injury may extended beyond the radiation field and causes death in approximately a third of patients and/or severe fibrosis overlapping the radiation field in the survivors. Patients with scleroderma undergoing breast irradiation have a significant increase in radiation-related complications including arm edema, chest wall necrosis, brachial plexopathy, pneumonitis, severe cutaneous fibrosis with retractions, and fistulae. When scleroderma patients with breast cancer are being considered for breast-conserving surgery, radiation is currently listed as a relative contraindication.[35,36]

While excessive radiation-associated toxicity in scleroderma may occur with high-dose radiation therapy (40–70 Gy), at lower radiation doses (18 Gy), scleroderma may be associated with radiation-related toxicities that are difficult to distinguish from disease exacerbation.[37] Similar to the use of TLI in RA and SLE, scleroderma has also been treated with TLI (1800 cGy divided 200 cGy 5 days a week).[37] owing to unexpected toxicity, this study was aborted early after enrolling only six patients (three patients in each of the control and treatment group). In the treatment group, two patients, despite lung shielding, suffered marked deterioration in pulmonary function and the third died from complications of gastrointestinal scleroderma. Therefore, acute TLI-related toxicity appears significantly worse in patients with scleroderma compared to normal patients or even patients with other autoimmune diseases such as SLE, or RA.

HSCT irradiation-based conditioning regimen in scleroderma

Binks *et al*[2] reported on the outcome of 41 patients with scleroderma following autologous HSCT. The data were generally focused on toxicity and skin scores. This was a retrospective analysis from 18 centers using seven different conditioning regimens from separate institutional protocols. Nine patients were treated with a TBI-based regimen developed at the Fred Hutchinson Cancer Center. In all, 11 patients (27%) died. Mortality was secondary to TBI-related pulmonary deaths, disease progression, or pre-HSCT scleroderma-related pulmonary artery hypertension (PAH). Cardiopulmonary deaths in patients with PAH appeared to be secondary to inability to accommodate transplant-related cardiac stress from hyperhydration, infection, anemia, and/or fever. There was no evidence of cyclophosphamide-mediated hemorrhagic myocarditis. While this retrospective review did not correlate toxicity with the conditioning regimen, as an overview of multiple phase I studies, this paper urges caution in using TBI in the conditioning regimen and concern over including patients with PAH in HSCT protocols. The Binks study defined pulmonary improvement or deterioration as a 15% increased or decrease, respectively, in either vital capacity (VC) or carbon monoxide diffusion capacity (DLCO). Although the study did not correlate conditioning regimen and pulmonary function, VC and DLCO improved in 16 and 9% and deteriorated in 24 and 39%, respectively.

Subsequently, McSweeney *et al*[3] reported the Seattle experience in using a TBI-based regimen in 19 patients including the 9 patients previously reported in the Binks manuscript. Four patients (21%) died. TBI-based HSCT regimens for malignancy commonly utilize 1200 cGy of TBI without undue toxicity. Owing to concerns over scleroderma-related radiation toxicity, the TBI regimen was dose reduced to 800 cGy (200 cGy BID). Despite radiation dose reduction, two of the first eight patients died of TBI-related interstitial pneumonitis at days 58 and 79 post-HSCT. For the last 11 patients, the study was, therefore, amended with lung shielding (97% shielding after the first dose of 200 cGy). In these last 11 patients, two died. One died from Epstein–Barr virus infection (day 64) and one from renal failure (day 123). The etiology of renal failure was not stated and therefore a TBI-related acceleration of scleroderma nephropathy cannot be ruled out. In the remaining nine patients, pulmonary function at the time of last disease evaluation remained clinically unchanged (less than 15% variation) in four patients but declined by more than 15% in five patients. In these five patients, the declines in DLCO and months since HSCT were: 57 to 41% (14.5 months), 84 to 60% (12.8 months), 47 to 28% (10.8 months), 69 to 49% (9.6 months), and 72 to 52% (7.5 months).[3] While skin scores improved, there was a high incidence of acute and persistent pulmonary disease progression related to therapy with TBI. These data suggest significant TBI-related pulmonary toxicity in scleroderma occurs despite lung shielding and validates the 1993 scleroderma TLI study with lung shielding that was terminated early due to significant toxicity including pulmonary deterioration.

Radiation sensitivity of scleroderma

The reason(s) for scleroderma's unique sensitivity to radiation is not understood. One explanation is that radiation predisposes to the same pathologic response as occurs in scleroderma. Radiation injury may be synergistic or additive to ongoing scleroderma-related vascular injury and tissue fibrosis. Scleroderma is a vasculopathy with loss of capillaries, telangiectasia, and excessive collagen deposition. These are the same histologic features as seen in late radiation injury.[38] Acute radiation sickness occurs as a result of apoptosis of rapidly proliferating cells in organ systems such as skin, intestinal mucosa, and bone marrow. Late radiation-induced injury which may not manifest for months or years after exposure can arise from impaired

vascular function manifest by obliteration of capillaries, collagen deposition, fibrosis, and telangiectasia.[38]

It is assumed that early endothelial damage and increased vascular permeability causes or initiates the late pathologic features of fibrosis, reduced blood flow, and capillary loss. For this reason, several investigators have studied the effect of radiation on vascular permeability and or blood flow in rodent models. Significant leakage across intestinal capillaries occurs at 500 cGy TBI with leakage threshold beginning at 250 cGy.[39] Major abdominal vessels are more radioresistant, but small vessels within the intestinal wall may be obliterated within 3 weeks after 1000 cGy and 12 months after 600 cGy.[40] Pulmonary capillary perfusion was reduced in rats for 1–5 months after 1000 cGy and did not recover to normal levels at doses >1250 cGy.[41] In mice, effective renal plasma flow continued to decline for up to 1 year after a single dose of 1100 cGy.[42]

Vascular permeability after radiation has almost never been evaluated in humans. However, Avioli *et al*[43] demonstrated a drop in patient's effective renal plasma flow after exposure to as little as 450 cGy. The severity of vascular damage induced by radiation appears to depend on species, organ, dose, and patient susceptibility.[38] Small vessels such as capillaries are more susceptible to lower doses of radiation-induced late injury compared to larger vessels.[38] Radiation at even very low-dose chronic exposure is associated with damage to the microcirculation.[38] Fingernail-bed capillary microscopy of 145 physicians such as radiation oncologists and interventional radiologists with exposure to ionizing radiation at levels under 5 rem/year was compared to 106 control subjects comparable in age, sex, smoking history, blood glucose, and family history of diabetes but not exposed to low-level work place radiation.[44] The differences were statistically significant with 91% of the control group *vs* 19% of the radiation-exposed group showing no capillary damage. Marked or severe capillary damage was demonstrated in 6% of the control and 36% of the radiation-exposed group. Capillary damage correlated with work-life duration (ie years exposure to radiation) in the radiation-exposure group but not with duration of employment in the control group. Since scleroderma is also a disease manifest by microcapillary damage on fingernail bed microscopy, exposure to even low doses of radiation in patients with scleroderma should probably be avoided unless otherwise necessary.

Current clinical results following radiation suggest that scleroderma patients have a lower threshold for radiation-related vascular damage, a toxicity that is indistinguishable from disease progression. The sensitivity of scleroderma to radiation may be due to already impaired vascular blood flow, in the same manner as diabetes-related vascular compromise predisposes to an increased risk of radiation injury.[45] Therefore, it is perhaps not surprising that scleroderma, a disease marked by endothelial injury, fibrosis, and capillary loss, is sensitive to radiation-induced vascular injury. Tumor growth factor-beta (TGF-β) is associated with radiation-induced fibrosis and is elevated in patients with scleroderma in whom radiation may further elevate TGF-β and exacerbate scleroderma-related fibrosis.[46] Alternatively, scleroderma may be an acquired radiation-sensitive disease. Hereditary ultraviolet light or radiation-sensitive disorders such as xeroderma pigmentosum, Cockayne Syndrome, ataxia telangiectasia, trichothiodystrophy, Bloom syndrome, Werner syndrome, and BCRA 1/BCRA 2 arise from mutations in DNA repair enzymes.[21] Some but not all DNA repair-defective states are associated with an increased risk of cancer.

The torsional stress induced by twisting or coiling of DNA that arises during transcription and replication is relieved by topoisomerases that generate transient DNA breaks and induce rotation followed by religation of the relaxed DNA strands.[47] Scl-70 antibodies bind to the active site of topoisomerase I and inhibit topoisomerase I activity, preventing DNA relaxation.[48–51] It remains unclear if scl-70 antibodies are pathologic or an epiphenomena.[52] However, antibodies to topoisomerase I (Scl-70) are specific for scleroderma and are associated with a higher risk of diffuse skin disease, pulmonary involvement, and a worse prognosis.[52,53]

Drugs that inhibit topoisomerase I activity such as camptothecin/topotecan are known radiosensitizers.[54–56] In terms of *ex vivo* tumor cell line cytotoxicity, the combination of topotecan and radiation is synergistic, not just additive.[54–56] The laboratory *ex vivo* radiosensitizing effect of topotecan has led to clinical trials of combined topotecan and localized radiotherapy for treatment of solid tumors. Although untested in laboratory cell lines, it is conceivable that scl-70 by inhibiting topoisomerase activity (similar to topotecan) could also lower the threshold of scleroderma patients to radiation-induced toxicity.

The future of HSCT for scleroderma

Owing to improved skin scores, a randomized phase III study of the TBI-based regimen for scleroderma is being advocated.[3] An alternative and more conservative interpretation of the current data is that the phase I studies have demonstrated a possible role for high-dose immune suppression but indicate that patients with PAH should be excluded from HSCT and that radiation (TBI) should continue to be viewed as a relative contraindication for scleroderma. Further preclinical research into scleroderma-related TBI hypersensitivity is needed before advocating a TBI-based transplant regimen. Such studies could include *ex vivo* radiation sensitivity of cell lines with and without exposure to scl-70 antibody and *in vivo* radiation toxicity studies in topoisomerase knockout mice.

The improvement in skin scores and health assessment questionnaire following HSCT and lack of documented pulmonary toxicity in patients without PAH who were treated with non-TBI regimens in Europe as well as some American centers would suggest that future scleroderma HSCT studies should use non-TBI-based regimens.[57–62] TBI is not an essential agent and can be easily replaced with agents that have similar or higher immune ablative potential but without myeloablation, acute and chronic exacerbation of vascular injury, risk of damage to organ stem cell compartments, hindrance of repair mechanisms, and compared to TBI minimal risk of late malignancies.

Autologous HSCT using TBI conditioning in other autoimmune diseases

Besides systemic sclerosis, TBI-based conditioning regimens have been used for HSCT of multiple sclerosis (MS)[63,64] and juvenile idiopathic arthritis (JIA).[65] In a single center study of progressive MS patients treated with a TBI-based regimen, continued neurologic decline occurred in a significant percentage of patients with high pretransplant disability scores.[63] Post transplant disease progression raised concerns that TBI may be contributing to or aggravating axonal degeneration, the primary pathophysiology behind progressive loss of neurologic function.[63] These concerns were again raised in a HSCT study for progressive MS using BEAM, a near myeloablative regimen of four alkylating agents.[64] In this study, an ongoing decrease in brain volume suggested that continued axonal atrophy progressed for 2 years of reported follow-up after HSCT despite suppression of active inflammation.[64] Consequently, TBI-based regimens are no longer being utilized as a component of MS conditioning regimens. Learning from the failure of TBI in progressive MS, autologous HSCT for MS is now being directed towards using nonmyeloablative regimens in patients with lower disability, active relapses, and active inflammation (gadolinium enhancement) on MRI imaging.[66]

TBI, dose reduced to 4 Gy, has been used to treat pediatric patients with JIA.[67] Since TBI did not induce higher response rates compared to non-TBI regimens, concerns over use of TBI in pediatric patients, especially in terms of growth and late malignancies, resulted in elimination of TBI from future autologous HSCT regimens for JIA.[67] In general, autoimmune diseases, despite significant morbidity, typically have a lower mortality than most malignancies. Unlike malignancies, immune-mediated diseases including scleroderma may go into spontaneous or conventional treatment-related remission. Therefore, due to the risk benefit of HSCT and unpredictability of a patient's natural history, conditioning regimens for autoimmune diseases need a greater emphasis on safety than regimens designed for malignancies. In a general review of autologous HSCT in 263 patients with autoimmune diseases, the treatment-related mortality of myeloablative conditioning using either TBI or busulfan-based regimens was four times higher than for non-TBI-based regimens with no advantage in terms of disease control.[68] It is, therefore, paradoxical that scleroderma, a disease in which radiation is considered a relative contraindication, is the only autoimmune disease in which a TBI based conditioning regimen is still being advocated.

Autologous stem cells are only a supportive transfusion. The therapeutic efficacy and toxicity is derived from the immune-suppressive conditioning regimen. Agents with disease-specific efficacy at standard doses, such as cyclophosphamide and ATG for patients with scleroderma,[69] should be dose escalated in the conditioning regimen. Conditioning regimens for malignancies were designed for myeloablation, not immune ablation, and for patients in which organ dysfunction was a contraindication not an indication for therapy. Rather than applying malignancy-specific regimens to patients with autoimmune disease, regimens should be designed taking into account the rationale for this therapy, that is immune ablation not myeloablation, as well as unique aspects of organ dysfunction and tissue toxicity for each autoimmune disease.

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