Introduction

Despite advances in diagnostic and treatment modalities, cancer has emerged as one of the leading causes of mortality worldwide. As per current statistics, it accounts for 14.1 million new cases and 8.2 million deaths per year globally. Approximately 32.6 million people in various countries are living with cancer. This high mortality can be attributed to various limitations in cancer treatment. Owing to uncontrolled multiplication, tissue invasion and hematogenous spread of cancer cells, early containment and surgical resection is challenging. Moreover, micrometastasis to distant areas of the body often occurs much before the first presentation of cancer. Malignancies involving an inaccessible site, vital organs or in an advanced stage are less amenable to surgical removal en masse and their management largely depend on nonsurgical approaches such as chemotherapy and radiotherapy. Till date, no single chemotherapeutic agent has shown an optimum curative response without major adverse effects. Cancer relapse and recurrence frequently occur even with the multidrug combination. Poor bioavailability at the site of cancer, lack of specific action on tumor cells along with low therapeutic indices are the major limitations of cancer chemotherapy. On the other hand, radiotherapy can be delivered locally directed at the cancer site. However, it is merely suppressive rather than curative. Radiotherapy is less likely to kill all cancer cells. It only controls the tumor progression temporarily. Furthermore, a subpopulation of cancer cells may develop radioresistance making it ineffective. Consequently, there is a need for developing new strategies to treat cancers effectively. TNFerade is a novel genetic medicine which combined the anticancer actions of tumor necrosis factor-α (TNF-α) and concurrent radiotherapy. In this review, the biological, pharmacological, and clinical aspects of TNFerade as an anticancer drug and its future prospects are discussed.

History of Development

Adenovector have been used widely in gene therapy to deliver specific genes to target cells. It has success rate exceedingly higher than that of liposomes and other vectors. The development of radiation responsive promoter-regulated recombinant protein expression system at the University of Chicago paved the path for TNF-α based cancer gene therapy. TNFerade was developed by GenVec, Inc. in collaboration with Varian Medical Systems. GenVec’s proprietary replication deficient adenovector was used to transfer human TNF-α gene.
with its promoter. It received Food and Drug Administration (FDA) approval for its phase I clinical trials in December 2000.

**TNFerade Biology**

TNFerade is a novel immunotherapeutic agent which delivers human TNF-α gene to cancer cells using replication-deficient adenoviral vector.\(^8\) TNF-α is a potent proinflammatory cytokine produced by macrophages, lymphocytes, and fibroblasts in response to the infection and inflammation.\(^{109}\) TNF-α suppression has been studied as an approach to develop anti-inflammatory agents.\(^{11,12}\) On the other hand, since it can induce apoptosis leading to tumor necrosis and regression, it has been considered as a candidate for cancer immunotherapy.\(^{13}\) However, TNF-α, when administered systemically can cause severe adverse effects that are capillary leakage, disseminated coagulopathy, acidosis, and shock.\(^{8,14}\) The GenVec’s proprietary adenovector of TNFerade is made replication deficient by deletion of E1 and E4 genes. Deletion of E4 gene also ensures no inhibition of host protein synthesis by this vector. Furthermore, E3 gene is nonessential for viral growth in vitro and it is removed in an attempt to accommodate 3 kb gene of TNF-α.\(^8\) Following ligation of a radiation inducible promoter, early growth response (egr-1) gene to cDNA of TNF-α, it is cloned in E1, E3, E4 - deleted human adenovirus serotype 5 (Ad5). This is propagated in 293 ORF6 cells for optimum yield of TNFerade.

**Mode of Action**

TNFerade is administered as an intratumoral injection and used in conjunction with radiotherapy or chemotherapy for optimum tumoricidal effect.\(^{15}\) The rationale behind this combination is the synergistic lethal action of TNF-α and radiotherapy/chemotherapy on cancer cells.\(^{14}\) The egr-1 gene promoter has a key role in controlling the expression of TNF-α in response to radiation. It is proposed that radiation-induced reactive oxygen species (ROS) binds to CC (A + T rich) 6GG elements (known as CArG boxes) of the promoter and induce expression of TNF-α. ROS generated by chemotherapeutic agents like cisplatin also produce the same effect.\(^{14}\) It is proposed that TNF-α radiosensitizes cancer cells and overcomes the limitations of radiotherapy, such as radioreistance and metastasis.\(^{8}\) Chemotherapeutic agents such as cisplatin and fluorouracil were also found to have synergy with TNFerade when concomitantly.\(^{16,17}\) Locally produced TNF-α damages tumor capillary endothelium, induces inflammatory cell infiltration in tumor interstitium and exerts a direct toxic effect on tumor cells.

**Pharmacokinetics**

TNFerade injected in locally binds with coxsackie/adenovirus receptor and are taken up by tumor cells where it causes radiation-induced translation of TNF-α.\(^8\) Peak expression of TNF-α was detected 3 weeks after the transfection.\(^{18}\) The TNF-α has minimum systemic distribution. In one study, TNF-α had the highest concentration in cancer site, while the blood level was minimum (15.6 pg/mL).\(^{19}\) The lymphoreticular system has a critical role in the elimination of the adenovector. Inflammatory cells, especially phagocytes such as neutrophils and macrophages engulf and degrade adenovector.\(^8\) Recruitment of these inflammatory cells is attributed to chemotactic cytokines. TNFerade itself accelerates its own metabolism. Interleukin-8 induced by the adenovector and TNF-α expressed by the tumor cells act as a chemoattractant for these phagocytes.\(^{106}\) In addition, Ad5 being immunogenic mounts an immune response which also facilitates its metabolism.\(^{17}\) Hence, this immune elimination is likely to be more prominent with faster kinetics on subsequent exposures.

**Toxicity Profile**

Evidence from animal studies and clinical trials suggests that TNFerade has a superior margin of safety with potent tumoricidal action.\(^8\) TNFerade is devised for local action specifically at the cancer site. However, it was found to cause both systemic and local toxicity. Necrosis and ulceration at the injection site were frequently detected on the autopsy of tested animals.\(^{19}\) Hematological alterations such as a decrease in red cells and hemoglobin, elevation of leucocyte and platelet counts have been noted even in the absence of concurrent radiation exposure.\(^{18}\) Results from the animal model suggest, its toxicity is dose-dependent (i.e. higher toxicity with larger doses) and synergistic with radiation. TNFerade alone as well as with radiation was well tolerated over a dosage range of 4 × 10⁹–4 × 10¹⁰ particle units (PU) in 20 nude mice with the intratumoral injection. In contrast, subcutaneous injection of TNFerade in BALB/c mice was associated with mortality (5%, 1 out of 20), especially when combined with radiotherapy (25%, 5 out of 20).\(^{19}\) In another animal study, although the systemic injection of TNFerade caused death of one experimental animal, there were no physiological or anatomical abnormalities and the peak levels of TNF-α in blood following intravenous administration of 1 × 10¹¹ PU TNFerade were 325 pg/mL and 260 pg/mL in BALB/c mice and nude mice, respectively.\(^{8,20}\)

Similar results were obtained in human patients enrolled in various clinical trials. Lower doses of TNFerade (4 × 10⁹–4 × 10¹⁰ PU) was found to be associated with minimal blood levels of TNF-α (1–8 pg/mL) and least adverse effects.\(^{21}\) Although, theoretically there is a risk of systemic toxicity and neighbouring tissue injury due to spill over of TNF-α in blood from the tumor, especially at high doses, no such events have been reported.\(^{22}\) With few exceptions, serious or drug-limiting toxicities (DLT) and adverse drug interactions were not observed in most studies. Seiwert et al. reported thrombotic events as DLT at the dose of 3 (4 × 10¹¹) PU.\(^{23}\) In another study, cholangitis and pancreatitis were prominent at 1 × 10¹² PU.\(^{17}\) However, mild adverse effects such as fatigue, fever, chills, flu-like symptoms, nausea, vomiting, and pain at the injection site were common.\(^{24}\)

**Clinical Trials**

Preclinical in vivo studies using animal models have demonstrated superior anticancer effects of TNFerade with minimum adverse outcomes. Various human cancers were used as xenografts in nude mice which were subjected to intratumoral TNFerade injection in conjunction with radiotherapy.\(^{15,16}\) TNFerade was found to have selective action on tumor microvasculature resulting in a delay in tumor growth.
and tumor regression.\[25\] TNF-\(\alpha\) also achieved a sustained level of the tumor without any spill over into plasma.

Clinical evidence of TNFerade therapy in cancer patients is available from 2001 onwards. Results of clinical trials which have evaluated various clinical parameters of TNFerade as an anticancer agent are listed in Table 1. The initial phase I trial data supports the use of the therapeutic combination of radiotherapy and chemotherapeutic agents with TNFerade, as these modalities had superior tumor control without any additional increase in toxicity. Chemotherapeutics such as cisplatin, 5-fluorouracil, hydroxyurea, gemcitabine, and capecitabine has been used successfully.\[16,21,24,27\] TNFerade has been used over a dosage range of \(4 \times 10^7\)–\(1 \times 10^{12}\) PU with 30–70 Gy concomitant radiation for wide variety of cancers namely cancers of pancreas, esophagus, rectum, breast, lung, skin, head-neck carcinoma, and soft tissue sarcoma.\[16,26,28-30\] Two studies reported that the tumor response to TNFerade was independent of histology.\[20,29\] Although phase I clinical trials had improved overall and progression-free survival of cancer patients, in a phase II clinical trial for locally advanced pancreatic cancer standard of care plus TNFerade has failed to demonstrate a significant increase in patient survival in comparison to standard of care alone.\[31\] However, it was safe and had no serious toxicity.

**Limitations**

In spite of promising results shown in earlier studies, GenVec discontinued phase III clinical trial of TNFerade trial in 2010. There are still many hurdles that have to be overcome before TNFerade gets FDA approval. While intratumoral injection of TNFerade is an easy and simple approach, it has its own inherent limitations. Owing to its nondiffusible nature, the effective tumoricidal effect of TNFerade is often restricted to a limited area near the needle track.\[32\] This is especially critical in case of large tumors. Although intratumoral injection is essential for its selective effect on cancer cells, this mode of administration precludes its systemic distribution. Hence, TNFerade is not suitable for control of metastasis and haematological cancers.\[33\] Furthermore, intratumoral TNFerade administration is challenging and often not feasible for cancers involving inaccessible sites. Although, there is no reported incidence, it may give rise to serious systemic toxicity in case of accidental spillage of from tumor into the blood. TNFerade requires radiotherapy for activation of its radiation-inducible promoter. However, synergistic toxicity has been noted with concomitant radiotherapy and chemotherapeutic agents with TNFerade.

### Table 1:

**Clinical parameters of TNFerade therapy**

| Type of study | Reference | Study group | Clinical outcome | Safety and toxicity |
|---------------|-----------|-------------|------------------|---------------------|
| Phase I       | Sharma et al., 2001\[23\] | Patients with locally advanced, recurrent, or metastatic solid tumors. Dose-escalation study | Out of 30 patients, 5 had CR, 9 PR, 8 MR, 4 SD and 4 PD Tumor response was independent of site and histology | Well tolerated No DLT |
| Phase I       | Mundt et al., 2004\[24\] | Patients with soft tissue sarcoma in the extremities | Out of 13 patients, 85% showed objective tumor responses (2 CR and 9 PR), and 1 had SD | Well tolerated Adverse effects: Fever (43.0%), chills (50.0%), fatigue (36.0%), flu-like symptoms (21.0%) |
| Phase I       | Senzer et al., 2004\[25\] | Patients with solid tumors | Out of 36 patients, 70% demonstrated objective tumor response | Well tolerated No DLT Adverse effects: Fever (22%), chills (19%), injection site pain (19%) |
| Phase I       | McLoughlin et al., 2005\[26\] | Patients (n=19) with advanced solid tumors | Tumor response was independent of histology | No DLT Both short-term and long-term safety is observed No DLT |
| Phase I       | Chang et al., 2012\[19\] | Patients (n=24) with locally advanced (stage II and III) resectable oesophageal cancer | Cisplatin and 5-fluorouracil with TNFerade were associated with long survival (median overall survival was 47.8 months) | No DLT Adverse effects: Fatigue (54%), fever (38%), chills (21%) nausea (29%), vomiting (21%), esophagitis (21%) |
| Phase I       | Seiwert et al., 2013\[27\] | Patients (n=14) with recurrent head and neck cancers | Response rate was 83.3%. Median survival was 9.6 months | TNFerade at an MTD of \((4 \times 10^{10})\) PU can be integrated with hydroxyurea and 5-fluorouracil DLT: Thrombotic events at a dose level of 3 \((4 \times 10^{10})\) PU |
| Phase II      | Hecht et al., 2012\[17\] | Patients with locally advanced pancreatic cancer | Out of 50 patients, one had CR, 3 PR, and 12 SD | DLT: Pancreatitis and cholangitis at \(1 \times 10^{12}\) PU dose |
| Phase III     | Herman et al., 2013\[31\] | Patients with locally advanced pancreatic cancer 187 patients received SOC +TNFerade, 90 patients were only on SOC | Median progression-free survival was 6.8 months for SOC + TNFerade and 7.0 months for SOC alone but not effective in prolonging survival | Both SOC+TNFerade and SOC arms had similar grade of toxicities |

DLT=Dose-limiting toxicities, MTD=Maximum tolerated dose, OR=Objective response, CR=Complete response, PR=Partial response, MR=Minor response, SD=Stable disease, SOC=Standard of care, PU=Particle units, TNF=Tumor necrosis factor, PD=Progressive disease
radiotherapy.\cite{9} TNFerade also has adenovector related risks. Theoretically, there is a chance that the replication deficient adenovirus may mutate and becomes replication competent with pathogenic potential, leading to serious infection.\cite{10} Furthermore, an immune response against adenovector may accelerate the metabolism of TNFerade on subsequent exposure. Hence, it may lack effective tumoricidal activity of repeated use.\cite{11}

**Future Prospects**

Despite the limitations and the incongruous result of phase III trial in pancreatic cancer patients, TNFerade has significance as an anticancer agent because of its wide applications. The novel radiation-inducible promoter and adenovector technology synergistically combines the benefits of TNF-α immunotherapy and radiotherapy without the systemic toxicity. Furthermore, a variety of tumors in accessible sites can be effectively treated irrespective of histological types.\cite{12} Intratumoral TNFerade injection precludes the need of isolated limb perfusion for the administration of chemotherapy in sarcomas and melanoma of extremities.\cite{13} TNFerade administration to inaccessible tumors has been carried out using percutaneous, endoscopic, and endoscopic ultrasound (EUS) guided approaches with success.\cite{14} These minimally invasive techniques obviate the difficulties of intratumoral administration of TNFerade in esophageal, gastric, rectal, bile duct, pancreatic, and lung cancers. EUS is especially important for pancreatic cancer. Preoperative TNFerade therapy does not interfere with tumor resection. On the contrary, it has been found to have a beneficial role as it results in tumor shrinkage.\cite{15}

**Conclusions**

TNFerade associates the anticancer actions of TNF-α and concurrent radiotherapy and also permits the spatial and temporal control of TNF-α expression by selecting the appropriate timing and area for radiotherapy. The development of newer minimally invasive interventional drug delivery approaches has also extended its scope, especially in pancreato-biliary, gastrointestinal, and lung cancers. However, further studies are needed to determine its therapeutic potentials.

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**Conflicts of Interest**

There are no conflicts of interest.

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