Electro-therapies for sarcomas

Abstract
Sarcomas are cancers that occur in soft tissues and bones that mostly affect teenagers and young adults. Although its occurrence is rare, the affect is huge-metastatic and deaths are imminent. This mini review reports the various aspects of sarcomas, including Ewing’s sarcoma and also discussed is the use of electrical pulse-based therapies, such as electrochemotherapy (ECT) and irreversible electroporation (IRE) for those patients who could not benefit from the standard therapies. Typically, 1200V/cm or 1000V/cm, 100µs, eight pulses are used for ECT and bleomycin and cisplatin are the two most commonly administered drugs. Lately calcium and other bio-compounds, such as mint are explored as anticancer drugs. In IRE, 80 pulses of 1000-1500V/cm, 100µs are used. In general electrotherapies offer an attractive alternative to current standard therapies when they are refractive.

Keywords: sarcoma, ewing sarcoma, soft tissue sarcoma, electrochemotherapy, irreversible electroporation

Abbreviations: ECT, electrochemotherapy; IRE, irreversible electroporation; GIST, gastrointestinal stromal tumors; ESFT, Ewing’s sarcoma family of tumors; ES, Ewing sarcoma; PNET, peripheral primitive neuroectodermal tumor; NSE, neuron-specific enolase; EP, electroporation, STS, soft tissue sarcoma

Introduction
Sarcomas are cancers of the bones and soft tissues. They are generally divided into soft tissue sarcomas (STS) and bone sarcomas based on their different mesenchymal origins and anatomical locations. They develop from cells that maintain the structure or cushion other organs in our bodies, including bone, cartilage, muscle, fat, and tendons. The different types of sarcomas are: a) Primary bone sarcomas, including Chondrosarcoma, Ewing Sarcoma, and Osteosarcoma. The various soft tissue sarcomas are: Angiosarcoma, Gastrointestinal Stromal Tumors (GIST), Kaposi Sarcoma, Leiomyosarcoma, Liposarcoma, Synovial Sarcoma, Undifferentiated Pleomorphic Sarcoma, Desmoid Fibromatosis and Pigmented Villonodular Synovitis. They are uncommon and make up only 1 percent of all adult cancers. However, in children and young adults of up to 30years age, its occurrence is higher; up to 3/100,000 happen and especially Ewing Sarcoma is experienced by teens. Since sarcomas are transported by blood, their spread is quicker and often secondaries are possible, compared to other cancers, such as carcinomas. About 80 percent of sarcomas begin in the body’s soft tissues (cartilage, muscle, fat, and tendons). The other 20 percent arise in the bones. Medical news today indicates that 60% of sarcoma starts in an arm or leg, 30% in abdomen or torso and 10% in neck or head. The greatest numbers of bone tumors are metastatic and spread to lung, colon, or breast.

Figure 1 shows different types of sarcomas including angiosarcoma, osteosarcoma, Ewing sarcoma, Chondrosarcoma, etc.

Ewing’s sarcoma
Ewing’s sarcoma is a high-grade osteolytic malignant neoplasm, described by James Ewing in 1921. It is the third most primary malignant bone tumor, after multiple myeloma and osteosarcoma. It
forms from a certain kind of cell in bone or soft tissue and may be found in the bones of the legs, arms, feet, hands, chest, pelvis, and spine as shown in Figure 2.\(^5\) It is classified as a group of small round blue tumor cells as shown in Figure 3, which includes neuroblastoma, alveolar rhabdomyosarcoma and lymphoblastic lymphoma.\(^6\) The Ewing’s sarcoma family of tumors (ESFT) includes classic Ewing sarcoma (ES), Askin tumor, and peripheral primitive neuroectodermal tumor (PNET).

**Figure 2** Occurrence of Ewing sarcoma in different parts of the body.\(^5\)

**Figure 3** (A) Histology of Ewing sarcoma. (B) The tumor cells of EFT show membranous expression of CD99/MIC2, and (C) nuclear positivity for antibodies against FLI1.\(^6\)
Morphologically, Ewing sarcoma is comprised of sheets of small round cells with a high nuclear to cytoplasmic ratio. The cells have weak eosinophilic cytoplasm containing glycogen and round nuclei with chromatin and little mitotic activity. Immunohistochemical analysis shows that 90% cases of Ewing sarcoma cells exhibit adhesion receptor CD99 or MIC2, usually related with the lymphoid cells mainly in leucocyte transmigration of the endothelium. Occasional rosette formation has been observed and does not produce any matrix. This tumor frequently undergoes necrosis and the residual viable cells show perivascular distribution. Rarely, these EFT tumor cells can be large with irregular nuclear membrane and prominent nucleoli.

Antibody against FLI1, which is present in the center of the nucleus of the tumor cells has been shown to be specific for EFT. Tumor cells may also express neuron-specific enolase (NSE), synaptophysin, and S-100 protein depending upon the degree of neurectodermal differentiation.

**Molecular genetics**

Sarcomas can be subdivided into two distinct classes, depending upon the genetic mutations. One class includes tumors having complex karyotypic abnormalities without a particular pattern and other class includes Ewing sarcoma comprises tumors with unique chromosomal translocations leading to specific fusion genes. Increasingly, Ewing family of tumors are being characterized by t(11;22)(q24;ql2) chromosomal translocation, this generated fusion of 50 segment of the EWS gene with the 30 segment of the ETS family gene FLI-1. This resultant EWS-FLI-1 fusion protein acts as an aberrant transcriptional activator as shown in Figure 4, which contributes to the development of ESFT by altering the expression of its target genes in a better cellular environment. Table 1 shows the EWS fusion types in Ewings and other sarcomas.

![Figure 4](image-url)  
Schematic representation of the t(11;22) (q24;q12) translocation resulting in the generation of the EWS–FLI1 type 1 fusion transcript.

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Gene fusion</th>
<th>Tumour time (% of tumours with this EWS gene rearrangement)</th>
</tr>
</thead>
<tbody>
<tr>
<td>t[11;22](q24,ql2]</td>
<td>EWS-FLI1</td>
<td>ESFT (85%)</td>
</tr>
<tr>
<td>t[21;22](q22,ql2]</td>
<td>EWS-ERG</td>
<td>ESFT (10%)</td>
</tr>
<tr>
<td>t[7;22](q22,ql2]</td>
<td>EWS-ETV1</td>
<td>ESFT (rare)</td>
</tr>
<tr>
<td>t[17;22](q12,ql2]</td>
<td>EWS-E1AF</td>
<td>ESFT (rare)</td>
</tr>
<tr>
<td>t[2;22](q33,ql2]</td>
<td>EVVS-FEV</td>
<td>ESFT (rare)</td>
</tr>
<tr>
<td>t[12;22](q13,ql2]</td>
<td>EWS-AFT1</td>
<td>Clear cell sarcoma</td>
</tr>
<tr>
<td>t[11;22](q13,ql2]</td>
<td>EWS-WT1</td>
<td>Desmoplastic small round cell tumor</td>
</tr>
<tr>
<td>t[9;22](q22,ql2]</td>
<td>EWS-CHN</td>
<td>Myxoid chondrosarcoma</td>
</tr>
<tr>
<td>t[12;22](q13,ql2]</td>
<td>EWS-CHOP</td>
<td>Myxoid liposarcoma</td>
</tr>
</tbody>
</table>
Electro-therapies for sarcomas

Electrochemotherapy and irreversible electroporation

The cell plasma membrane of human cells, is a microscopic bilayer of protein and phospholipids, with hydrophobic and hydrophilic ends. This structure allows only a controlled flow of selected ions and molecules through membrane proteins working as ion channels while regulating a concentration of ions on both of its sides (extra- and intra-cellular). This gives rise to a potential, which allows the cell membrane to function as a leaky dielectric, having both capacitive and conductive properties due to charge accumulation and conduction. In general, large, charged and polar chemotherapeutic molecules are impermeable or poorly permeable across the membrane, due to its structure.

The local application of short duration, high-intensity electrical pulses across the cell membrane results in the charge accumulation and increases the Vm. When the Vm increases beyond the threshold (v0.5V), there is a several-fold enhancement of electric field, leading to the pore formation. This phenomenon, known as electroporation (EP) can enhance the uptake of external molecules up to 1000 times. When EP is applied towards the uptake of chemotherapeutics, this non-surgical, physical procedure is called Electrochemotherapy (ECT).

ECT is gaining momentum as an alternate modality for advanced, inoperable, radiation and chemo-resistant tumors, including cutaneous and subcutaneous metastases in Europe. Electroporation generally depend upon the intensity of the electric field, the duration of each pulse, the number of pulses, and the interval between them. Depending upon the magnitudes of these parameters, we can have different effects on the cell membrane: no changes on the cell membrane, temporary opening of the cell membrane after which cell can still survive (reversible electroporation), and permanently open the cell membrane and the cell dies after (irreversible electroporation). Reversible electroporation is used in electrochemotherapy, wherein low dose chemotherapics and macromolecules, are administered into the targeted area.

Electrodes are placed around the tumor area, generating reversible permeabilization that allows substances to pass the cell membrane and into the cytoplasm. Irreversible electroporation (IRE) is that which causes permanent permeabilization of the cell membranes and the consequent loss of cell homeostasis, when an electrical field is applied to cancer cells. Irreversible electroporation (IRE) is that which causes permanent permeabilization of the cell membranes and the consequent loss of cell homeostasis, when an electrical field is applied to cancer cells. Irreversible electroporation (IRE) is that which causes permanent permeabilization of the cell membranes and the consequent loss of cell homeostasis, when an electrical field is applied to cancer cells.

IRE for sarcomas

Irreversible electroporation is gaining momentum as a nonthermal tissue ablation technique in treating sarcomas. Steinbrecher et al. report about using IRE as a curative treatment for Ewing’s sarcoma. In this case, the patient, a 9 year old girl could not undergo surgery or radiation due to the high risk of sacral nerve plexus destruction and paralysis. Four cycles of salvage chemotherapy using vincristine, irinotecan, temozolomide and zolendronic did not reduce the tumor size. Since surgery and radiation is not possible in this patient as mentioned above due to the risk of neural damage and possible paralysis, and chemotherapy is not working, IRE was chosen as the best option for a curative paradigm. The patient went through IRE following the fifth cycle of chemotherapy. Using CT guidance and five 11-gauge coaxial trans- osseous needles, IRE was performed under CT guidance. Two IRE probes were used and the procedure was completed with no complications—there were no loss of lower extremity, bowel, or bladder motor strength or sensation. Follow-up using MRI and PET-CT showed a complete radiologic response. Three years later, patient remains in continuous clinical remission.

Both single electrode probe and two electrode probes (Figure 5) are used for IRE for treating a 7-year old spayed female Labrador retriever. It had a five year history of degenerative coxofemoral joint disease, causing bilateral pelvic limb lameness. The voltage applied varied from 1000 to 1500V, with the exception of one, where 800V was used. 80 pulses were applied each time, with a pulse length of 100µs, except in one case, where 70µs was used.

The main advantage of IRE in comparison to other ablation techniques is its non-thermal nature. Due to localization of the applied electric field structures such as collagen and elastin are not damaged due to the treatment. In addition, the electrical properties of cancer cells are more conducive to electrical pulse-based therapy.

than normal cells.44 The cancer cells are more conductive, has more sodium, and lower membrane potential—all make the cancer cells more susceptible to the electrical pulses and the electric field than the normal cells. In addition, electric field is inversely proportional to distance squared, proximity effect is minimum or nil. The literature search indicates that this is a first treatment of ES using IRE.

Figure 5 Single and two probe electrodes used for IRE.

Summary

a. When standard therapies, such as surgery, radiation, and chemotherapy do not work for the various sarcomas, we need alternate therapies.

b. Electrical pulsed-based therapies, such as ECT and IRE offer effective and economical alternatives for those patients with recurrent, inoperable ES, STS, and other sarcomas, especially, in close proximity to vital structures could be effectively treated using IRE as a curative paradigm.

c. Electrochemotherapy is promising for recurrent and inoperable soft tissue sarcomas and Kaposi sarcoma.

d. Typically eight 1200V/cm, 100µs pulses at 1Hz or 5kHz are used for electrochemotherapy.

e. Eighty 100µs pulses at intensities of up to 1500V/cm are used for IRE.

f. The 100s of clinical trials of a number of sarcomas, including STS and ES using chemo drugs indicate that there is potential for alternate therapies, such as ECT and IRE, when the standard therapies do not work.

Acknowledgments

None.

Conflicts of interest

The authors declare there is no conflict of interest.

References

1. What you should know about sarcoma.


