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Electrochemotherapy achieved prolonged local control of mammary adenocarcinoma in an Asian elephant (*Elephas maximus*)

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Objective
Tumors in elephants present substantial therapeutic challenges because of their large size, anatomical complexity, and the risks associated with surgical intervention. Targeted electrochemotherapy (ECT) has emerged as an effective local treatment modality in both human and veterinary oncology. However, its application in exotic species has been rarely reported.

Animal
An adult female Asian elephant (*Elephas maximus*) housed at the El Paso Zoo in Texas.

Clinical Presentation, Progression, and Procedures
The elephant was diagnosed with a large solid tumor considered unsuitable for conventional surgical management. The animal underwent 6 cycles of ECT consisting of intratumoral bleomycin administration followed by the delivery of electric pulses according to established veterinary ECT protocols.

Treatment and Outcome
Treatment was well tolerated, with no clinically apparent adverse effects. Effective tumor reduction was observed in response to the first 3 ECT treatments, resulting in sustained local tumor control for more than 1 year. Electrochemotherapy was feasible, safe, and clinically effective in achieving prolonged local tumor control in an Asian elephant with mammary adenocarcinoma.

Clinical Relevance
To the authors' knowledge, this is the first report describing the use of ECT in an elephant for the treatment of a large solid tumor. The favorable clinical outcome and absence of significant adverse effects support the feasibility and potential utility of ECT as a minimally invasive treatment option for neoplastic diseases in elephants and other exotic species. Furthermore, this case exemplifies a reverse translational approach in which therapeutic strategies established in human oncology are successfully adapted to veterinary medicine.

Keywords: targeted electrochemotherapy, oncology, adenocarcinoma, exotic, elephant

Cancer represents an important cause of morbidity and mortality in both domestic and exotic animal species, often requiring multimodal therapeutic approaches to achieve optimal disease control. Although surgery remains the primary treatment for many localized neoplasms, its application in exotic species can be limited by anatomical, physiological, and logistical constraints, as well as by the risks associated with anesthesia and postoperative management. Alternative local treatment modalities are therefore of particular interest in zoological medicine and wildlife oncology.

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Electrochemotherapy (ECT) is a local antitumor treatment that combines the administration of cytotoxic drugs, most commonly bleomycin or cisplatin, with the application of short, high-voltage electric pulses that transiently increase cell membrane permeability through reversible electroporation. This process enhances intracellular drug uptake, thereby increasing cytotoxic efficacy while limiting systemic exposure. Over the last 2 decades, ECT has become an established treatment modality for cutaneous and subcutaneous tumors in human oncology and has demonstrated high objective response rates with minimal adverse effects.[1–3]

In veterinary medicine, ECT has been successfully applied to a variety of spontaneous tumors in dogs, cats, horses, and other species, including soft tissue sarcomas, mast cell tumors, squamous cell carcinomas, melanomas, and mammary tumors.[4–7] The technique has gained increasing acceptance because of its minimally invasive nature, favorable safety profile, and demonstrated efficacy in achieving durable local tumor control, particularly in patients for whom surgery or radiation therapy is either not feasible or would be expected to result in substantial treatment-related morbidity.[8]

Mammary gland tumors are among the most common neoplasms in women and represent a major focus of clinical research in human oncology. In recent years, ECT has emerged as a valuable treatment option for primary and recurrent breast cancer lesions, particularly in patients who are poor surgical or radiation therapy candidates or have exhausted conventional treatment options. Clinical studies[3,9,10] have demonstrated effective local tumor control, symptom palliation, and preservation of tissue function with limited toxicity.

Neoplasia appears to occur less frequently in elephants than would be expected based on their body size and longevity, a phenomenon often cited as an example of the Peto paradox. This is based on the idea that if every cell has some chance of becoming cancerous, large, long-lived organisms should have an increased risk of developing cancer compared with small, short-lived organisms. The paradox consists in the lack of correlation between body size and cancer risk. Studies suggest that elephants possess multiple copies of the tumor suppressor gene *TP53*, which may contribute to enhanced cancer resistance mechanisms.[11] Nevertheless, neoplastic diseases do occur in both African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants and can present substantial therapeutic challenges because of the animals' large body size, anatomical characteristics, and the risks associated with anesthesia and surgical intervention.

The management of tumors in elephants is particularly complex when lesions are large, poorly accessible, or located near critical anatomical structures. Surgical excision may be associated with significant perioperative risks, including anesthetic complications, hemorrhage, prolonged recovery, and wound-healing difficulties. Consequently, minimally invasive treatment alternatives capable of providing local tumor control are highly desirable in this species.

This clinical report describes the use of ECT in a 53-year-old female Asian elephant (*E maximus*) with a large cutaneous tumor that was considered unsuitable for surgical management because of its size and location. To the authors' knowledge, this is the first published report documenting the successful application of ECT in an elephant, demonstrating the feasibility, safety, and potential clinical utility of this technique in the management of large neoplastic lesions in megavertebrate species.

Methods

Histopathologic examination

Formalin-fixed, paraffin-embedded tissue sections (4 µm) were stained with hematoxylin and eosin for routine histopathologic examination. It was performed according to the protocol previously described by our group for canine transitional cell carcinomas.[12] Briefly, sections were incubated with a polyclonal anti-human epidermal growth factor receptor 2 (HER2/neu) antibody (Dako) at a dilution of 1:250 in Tris-buffered saline for 1 hour at room temperature. At the completion of the immunohistochemical analysis, the tumor showed no detectable HER2/neu immunoreactivity, indicating the absence of HER2 overexpression.

Anesthesia

All ECT procedures were performed under general anesthesia under the supervision of the El Paso Zoo veterinary team. Because the elephant was a geriatric, approximately 8,000-lb (3,600-kg), 53-year-old Asian elephant (*E maximus*), minimizing anesthetic risk was a major consideration in treatment planning. Consequently, the duration of anesthetic episodes was limited, and treatment intervals were adapted to balance oncologic benefit with patient safety. Although the total number of episodes was unusual for an elephant and not anticipated at the outset of treatment, the elephant's stability during and recovery following each procedure allowed consideration of additional sessions in response to tumor behavior.

Before each procedure, the elephant voluntarily entered a custom-designed elephant restraint chute as part of her routine husbandry training. A sedative dose of 200 mg azaperone was administered IM by hand injection. Anesthesia was induced with 9 mg etorphine IM by hand injection. The hydraulic restraint system gradually secured the animal in a standing position during anesthetic induction and subsequently tilted to allow controlled transition into lateral recumbency, minimizing the risk of injury associated with uncontrolled collapse. This approach facilitated safe positioning and access to the mammary mass while reducing stress for the animal and personnel.

General anesthesia was maintained for approximately 90 minutes during each treatment session. Etorphine was supplemented as needed by 0.5- to 1-mg IV boluses for induction or etorphine continuous rate infusion for maintenance. The elephant was intubated with a 35-mm inner-diameter endotracheal tube and ventilated using a megavertebrate demand ventilator. An IV catheter was placed in a caudal

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ear vein for medication administration. Throughout anesthesia, parameters including temperature, oscillometric blood pressure, end-tidal CO_2, pulse oximetry, ECG, and venous and arterial blood gases were monitored by veterinarians experienced in elephant medicine, and supportive care was provided until recovery. Following completion of the ECT procedure, anesthesia was reversed with naltrexone at 500 mg IV and 500 mg IM. The elephant recovered with positional support within the restraint system before safely returning to an unsupported standing position. No anesthesia-related complications were observed during any of the treatment sessions.

The large body size of the patient and the risks associated with repeated general anesthesia influenced the treatment schedule. Unlike the short treatment intervals commonly adopted for companion animals undergoing ECT, sessions were intentionally spaced over longer periods to minimize cumulative anesthetic risk while maintaining local tumor control.

Electrochemotherapy

Electrochemotherapy was performed using a Cliniporator Vite pulse generator (IGEA). Bleomycin sulfate was administered by intratumoral injection to achieve homogeneous distribution throughout the lesion. Approximately 1 minute after drug administration, electric pulses were delivered using single stainless-steel needle electrodes (diameter, 1.2 mm; IGEA) inserted directly into the tumor tissue. Four electrodes were positioned according to a predefined treatment grid to ensure adequate electric field distribution throughout the tumor volume and surrounding margins. Electrode repositioning was performed repeatedly to treat overlapping regions until the entire gross tumor volume and clinically identifiable margins had been covered.

The Cliniporator Vitae system automatically recorded all treatment parameters, including electrode configuration, interelectrode distance, applied voltage, pulse duration, pulse number, and delivered current for each application. Treatment data were reviewed after each procedure to verify appropriate pulse delivery and tissue electrical conductivity. Following completion of ECT, the treated area was visually inspected for evidence of hemorrhage or immediate complications before recovery from anesthesia. Clinical follow-up consisted of serial physical examinations and photographic documentation to evaluate changes in tumor dimensions, tissue appearance, and local treatment-related adverse events.

Euthanasia

For euthanasia, the El Paso Zoo Veterinary team follows strict protocols outlined by AVMA to ensure a peaceful, pain-free transition. Because a sudden fall can cause massive trauma or dangerous distress, the process was carefully staged into 2 distinct phases: the elephant was first anesthetized as previously described. A large-bore catheter was carefully placed into a prominent vein in the animal's ear. Then, the terminal agents (300 mg azaperone and 12 mg etorphine in 177 mL of solution) were administered.

Results

Histopathologic diagnosis

In October 2016, a 53-year-old female Asian elephant (*E maximus*) was diagnosed with a malignant mammary gland neoplasm involving the right mammary region. Histopathologic examination of biopsy specimens revealed a densely cellular epithelial neoplasm composed of tubulopapillary structures, solid sheets, and acinar arrangements supported by scant fibrovascular stroma and surrounded by minimal collagenous connective tissue **(Figure 1)**.

The tubules and papillary projections were lined by 3 to 6 layers of neoplastic polygonal epithelial cells with indistinct cell borders, scant eosinophilic cytoplasm, and oval to polygonal nuclei. Nuclear chromatin was finely stippled, and nuclei contained 1 to 6 prominent nucleoli. Moderate anisocytosis and anisokaryosis were observed. Mitoses were also frequently observed. Based on the histomorphological features, the lesion was diagnosed as a mammary adenocarcinoma.

To further characterize the tumor, immunohistochemical staining for HER2/neu was performed. The tumor at the end of the immunohistochemistry experiment showed no staining (data not shown).

Electrochemotherapy procedure

Because of the tumor's large size and anatomical location **(Figure 2)**, combined with the geriatric status of the elephant, surgical excision was considered to carry substantial anesthetic and surgical

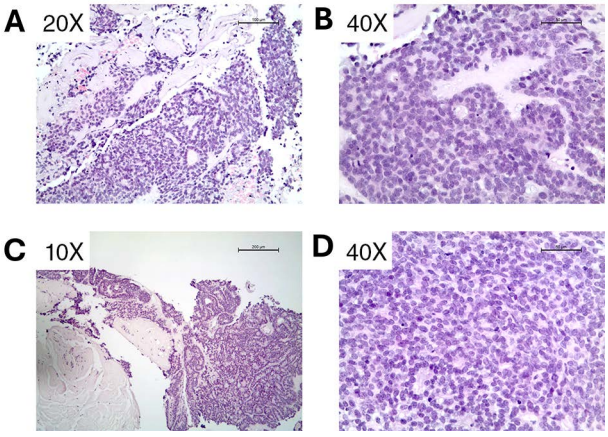


Figure 1—Histopathologic features of the mammary adenocarcinoma diagnosed in a 53-year-old Asian elephant (*Elephas maximus*). Representative hematoxylin and eosin-stained sections showing the neoplasm at different magnifications. A—Low-power view (20X) demonstrating infiltrative epithelial proliferation arranged in tubulopapillary structures within scant fibrovascular stroma. Bar = 100 µm. B—High-power view (40X) showing multilayered neoplastic epithelial cells with moderate anisocytosis and anisokaryosis, oval to polygonal nuclei, finely stippled chromatin, and prominent nucleoli. Bar = 50 µm. C—Low-power view (10X) illustrating the interface between the neoplasm and adjacent mammary tissue. Bar = 200 µm. D—High-power view (40X) highlighting the solid growth pattern of the neoplastic cells. Bar = 50 µm.

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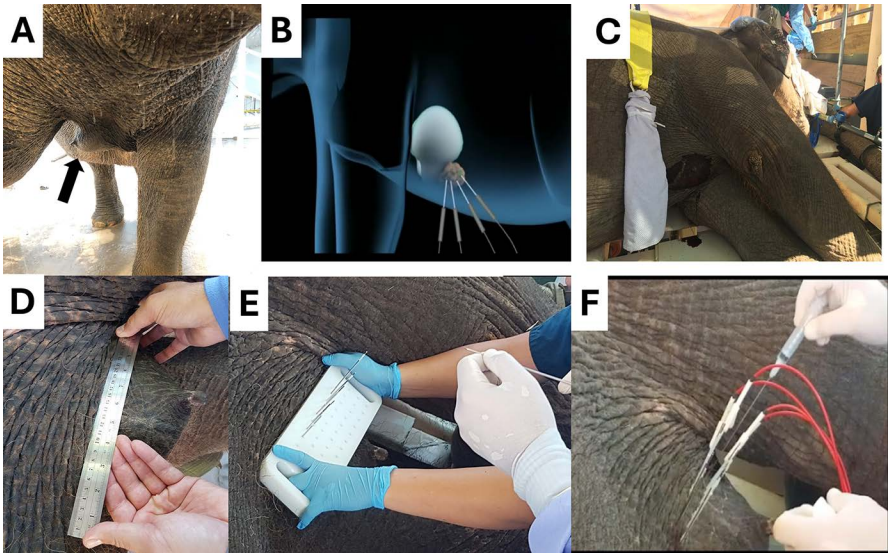


Figure 2—Electrochemotherapy procedure in a 53-year-old Asian elephant (*Elephas maximus*). A—Large mammary mass before treatment. The arrow indicates the tumor mass. B—Schematic representation of 4-needle electrode placement within the tumor to ensure adequate electric field distribution. C—Positioning of the elephant under general anesthesia for electrochemotherapy. D—Measurement of the tumor with a ruler. E—Positioning of electrodes with a dedicated grid. F—Delivery of electric pulses using the Cliniporator Vitae system after intratumoral administration of bleomycin.

risks. Targeted ECT was therefore selected as a minimally invasive alternative for local tumor control. Given the considerable tumor dimensions and the thickness of the overlying skin, standard fixed electrodes commonly used for human ECT, including parallel-row or hexagonal configurations with electrode lengths typically ranging from 10 to 40 mm, were considered unsuitable. Longer electrodes, more appropriate for the treatment of deep-seated lesions, were therefore selected. All ECT sessions were performed under general anesthesia to allow adequate exposure and accessibility of the tumor and to ensure safe and accurate electrode placement. At treatment initiation, the tumor volume was estimated to be approximately 246 cubic inches (4,030 cm^3). Because of the exceptionally large size of the lesion, a staged treatment strategy involving multiple ECT sessions was planned to maximize tumor coverage while limiting the duration and invasiveness of each individual procedure and maintaining overall procedural safety.

Electrochemotherapy was delivered using a Cliniporator Vitae device (IGEA). Single-needle stainless steel electrodes (diameter, 1.2 mm; length, 20 to 24 cm) were inserted directly into the tumor tissue. Electrodes were positioned around the lesion according to a predefined treatment grid to ensure homogeneous electric field distribution and adequate coverage of both the gross tumor volume and surrounding margins (Figure 2).

Bleomycin (15 to 30 mg) was administered intratumorally approximately 1 minute before pulse delivery (Figure 2). Electrical treatment consisted of 8 consecutive square-wave pulses delivered at 400 to 700 V, pulse duration of 100 µs, and frequency of 5,000 Hz. Because complete treatment of tumor margins is essential to address potential microscopic disease and irregular tumor architecture, multiple overlapping applications were performed during each treatment session to encompass the largest possible tumor volume.

The Cliniporator Vitae system automatically recorded all treatment parameters, including electrode configuration, interelectrode distance, pulse amplitude, pulse duration, and number of pulses delivered. A representative treatment profile is shown in **Figure 3**.

The elephant underwent a total of 6 ECT treatment cycles between October 2016 and February 2020. The procedure was well tolerated, and no clinically significant local or systemic adverse effects were observed during or after treatment.

Clinical outcome

As a general observation, in the first week after each treatment, transient swelling was observed, followed by gradual resolution by 3 weeks post-ECT **(Figure 4)**. Following resolution of posttreatment swelling, a progressive reduction in tumor size was observed, with a maximal reduction (54%) at day 51. However, the tumor progressed again reaching the original size at day 112. At day 182, the tumor grew to 650 cubic inches (10.652 cm^3, ie, 1.6-fold the starting volume), and a new treatment was performed. An immediate reduction was observed until day 298 (70% reduction after the second ECT). To further prolong the effect, a third ECT was performed at that time point, followed by the fourth at day 330. However, successive ECT treatments resulted in no reduction but sustained local disease control (not shown). All clinical examinations documented a marked decrease in tumor volume and improvement in local tissue condition without evidence of treatment-related complications.

In November 2020, approximately 4 years after the initial diagnosis, renewed tumor progression was observed, characterized by accelerated local growth. Given the extent of the disease, the elephant's demonstrated resiliency to previous anesthetic episodes, and the limited remaining therapeutic options, surgical debulking was elected as a salvage procedure. Although surgery temporarily improved the animal's

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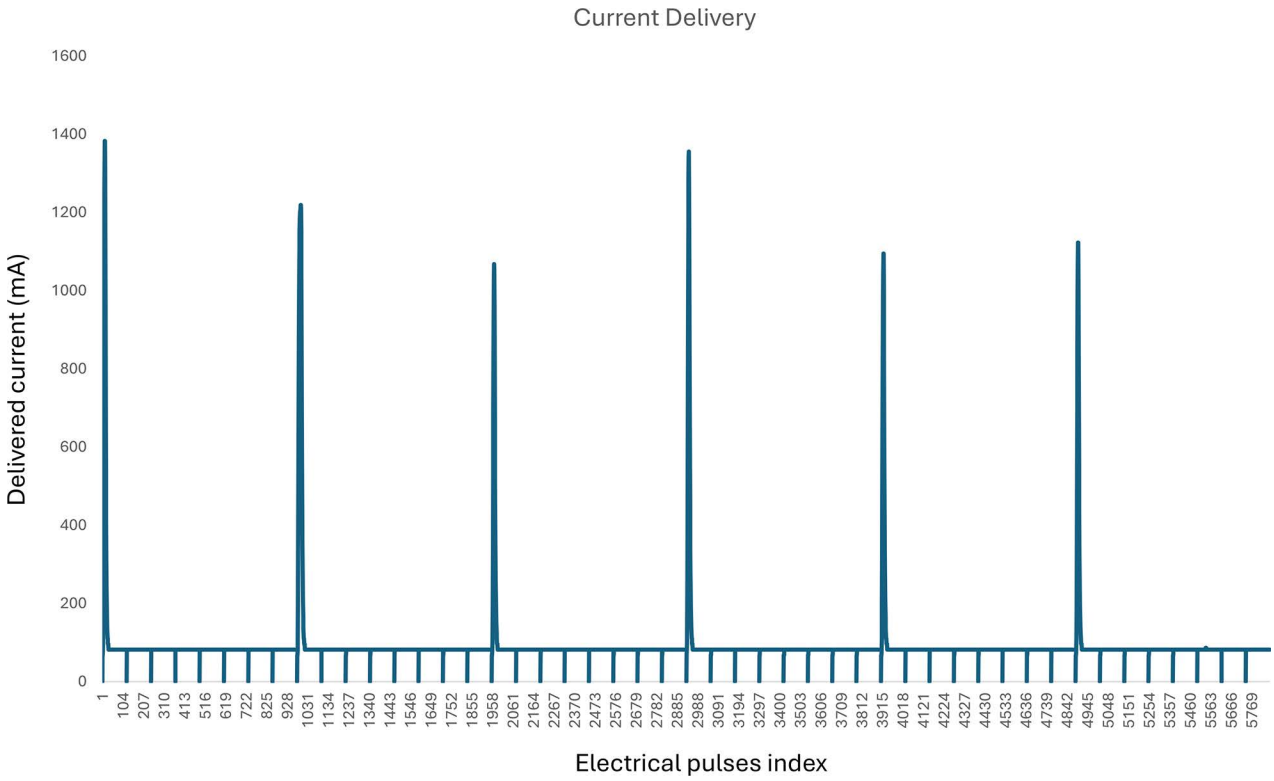


Figure 3—Representative electrical pulse delivery during electrochemotherapy. Current profile automatically recorded by the Cliniporator Vitae pulse generator during one representative treatment application, demonstrating stable current delivery throughout the pulse sequence. The x-axis represents the sequence of electrical pulses delivered by the electroporation device with each bar corresponding to one recorded pulse. The y-axis shows the current measured during each pulse, expressed in milliamperes.

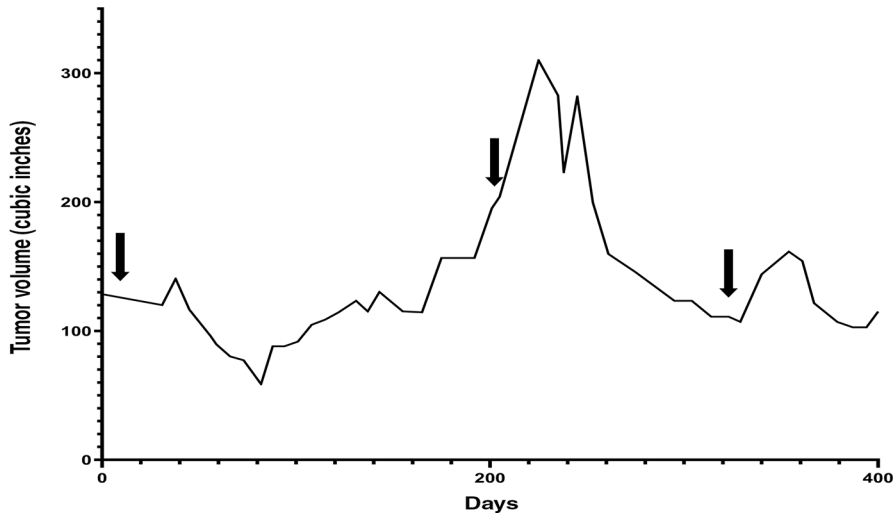


Figure 4—Tumor volume during electrochemotherapy treatment. Changes in tumor volume (cubic inches) over time following serial electrochemotherapy treatments. Vertical dashed lines indicate the timing of each treatment session. Progressive reduction in tumor volume was observed after each treatment cycle, resulting in prolonged local tumor control before subsequent disease progression.

comfort and quality of life, complete tumor excision was not achievable because of extensive local tissue involvement. Residual neoplastic tissue subsequently progressed, leading to local recurrence and complications during wound healing. As quality of life continued to decline and no further therapeutic interventions were considered feasible, euthanasia was elected. Overall survival from initial diagnosis was approximately 4 years.

Discussion

This clinical report describes, to the authors' knowledge, the first documented use of targeted ECT for the treatment of a mammary adenocarcinoma in an Asian elephant (*E maximus*). The treatment was well tolerated, resulted in substantial tumor reduction, and provided durable local tumor control for more than 1 year without clinically apparent adverse effects. These findings support the feasibility of ECT

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as a minimally invasive treatment option for large neoplastic lesions in elephants and potentially other megavertebrate species.

Management of neoplasia in elephants presents unique clinical challenges. Surgical excision, although considered the standard treatment for many localized tumors, may be associated with substantial anesthetic and perioperative risks because of the animal's size, specialized physiology, and logistical constraints associated with prolonged recumbency and postoperative care. Similar concerns have been reported in previous descriptions of surgical procedures performed in elephants, where anesthesia, wound management, and recovery represented major determinants of clinical outcome.[13,14] In the present case, surgical excision was initially considered too high risk based on the geriatric status of the elephant, the tumor size, and the anatomic considerations of the mammary gland including large vascular supply. The application of radiation therapy was also impractical because of the considerable logistical challenges associated with patient transport and the physical limitations of radiation therapy facilities. All these factors prompted consideration of an alternative local treatment strategy.

Electrochemotherapy combines reversible electroporation with local or systemic administration of cytotoxic agents, most commonly bleomycin, resulting in a marked increase in intracellular drug uptake and enhanced antitumor activity.[2] Over the last 2 decades, ECT has become an established treatment modality for cutaneous and subcutaneous tumors in human oncology and has demonstrated objective response rates exceeding 70% in several tumor types.[1,3] Similar results have been reported in veterinary medicine, where ECT has been successfully applied to spontaneous tumors in dogs, cats, horses, and other species, often providing durable local control with minimal morbidity.[4,5,7]

Published experience with ECT in exotic species remains limited. Nevertheless, reports[15] involving reptiles, birds, and zoo animals suggest that the biological principles underlying electroporation are broadly conserved across taxa and that ECT can be adapted to species with markedly different anatomical and physiological characteristics. The present case substantially extends this concept by demonstrating the practical applicability of ECT in one of the largest terrestrial mammals, requiring adaptation of electrode placement and treatment planning to accommodate an exceptionally large tumor volume.

One notable aspect of this case was the absence of clinically significant adverse effects despite repeated treatment sessions and the large treatment field. A transient period of local swelling was typically observed to peak at 1 week and resolve within 3 weeks of treatment. Discomfort was well managed with analgesics and did not impact patient mobility or appetite. This observation is consistent with the favorable safety profile reported in both human and veterinary patients, where adverse events are generally limited to transient local inflammation, oedema, or discomfort.[4,5,7] The ability to repeat ECT procedures over time without cumulative systemic toxicity is particularly relevant in species such as elephants, for which therapeutic options are often limited.

An important consideration arising from this case is the potential role of ECT as a neoadjuvant treatment for large or anatomically challenging tumors in elephants and other megavertebrate species. In patients for which primary surgical excision is associated with substantial anesthetic or perioperative risk, ECT may provide effective initial local tumor control and progressive tumor debulking, thereby reducing tumor volume before surgery. Such an approach could potentially improve surgical feasibility, decrease operative morbidity, and allow the veterinary team to gain experience with repeated anesthetic management before undertaking a major surgical procedure. Although this hypothesis cannot be confirmed from a single case, the clinical course observed in this elephant suggests that integrating ECT into a multimodal treatment strategy may represent a valuable option for selected patients with advanced localized neoplasia. Interestingly, this case also contributes to the growing field of comparative oncology. Although elephants are widely recognized for their relatively low cancer incidence, a phenomenon frequently referred to as the Peto paradox, neoplastic diseases remain an important clinical concern in aging captive individuals.[11] The remarkable cancer resistance observed in elephants has been linked to expansion of the *TP53* gene repertoire and enhanced DNA damage responses, yet these mechanisms do not confer complete protection against tumor development. The occurrence of mammary adenocarcinoma in the present case highlights the need for effective treatment strategies even in species characterized by reduced cancer susceptibility.

From a translational perspective, this report represents an example of reverse translational medicine. Electrochemotherapy was originally developed and validated in human oncology before being successfully adapted to veterinary patients. Knowledge gained from its application in domestic species subsequently facilitated implementation in a zoological setting. Such bidirectional exchange of knowledge between human and veterinary medicine is a cornerstone of comparative oncology and may accelerate the development of innovative therapeutic approaches across species.[16] The scientific and educational value of this case was further underscored by its inclusion in the National Geographic Wild television series *Animal ER*, which helped disseminate the application of ECT in zoological medicine to a broader audience.

This report has several limitations. As a single-case report, definitive conclusions regarding efficacy, survival benefit, or optimal treatment protocols cannot be drawn. In addition, objective assessment of treatment response was based primarily on clinical measurements rather than advanced imaging modalities. Moreover, due to complex logistics, we could not characterize the tumor at the molecular level. We cannot exclude the possibility that the lack of HER2 overexpression detected by immunohistochemistry

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reflects limited cross-reactivity of the antibody with the elephant HER2 receptor rather than the true absence of HER2 expression. Future studies involving additional zoological patients will be necessary to establish standardized treatment protocols, determine optimal dosing strategies for large-bodied species, and evaluate long-term oncologic outcomes.

In conclusion, ECT was feasible, safe, and clinically effective in achieving prolonged local tumor control in an Asian elephant with mammary adenocarcinoma. The favorable response observed in this case suggests that ECT may represent a valuable therapeutic option for elephants with tumors that are not amenable to conventional treatment modalities. Further investigation is warranted to define the role of ECT within the multidisciplinary management of neoplastic diseases in exotic and zoological species.

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None reported.

Disclosures

Drs. Conforti and Aurisicchio are employees at Evvivax. The biotech is developing vaccines based on electrogene transfer. Dr. Impellizeri is the founder of Veterinary Oncology Services and utilizes electrochemotherapy to cure pets.

Dr. Impellizeri served as Guest Editor for this *JAVMA* Supplemental Issue and was not involved in the editorial evaluation of or decision to accept this article for publication.

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