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Liquid Nitrogen-Recycled Autograft Augmented with a Non-Vascularized Fibular Strut for Refractory Pediatric Osteosarcoma: A Case Report of the Cryo-Immunological Reconstruction

Mujjadid Idulhaq[1], Khrisna Purwa Satyaksa[2*], Rachmad Faisal[2]

[1]Department of Orthopedics and Traumatology, Faculty of Medicine, Universitas Sebelas Maret/Prof. Dr. R. Soeharso Orthopedics Hospital, Surakarta, Indonesia

[2]Resident, Department of Orthopedics and Traumatology, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia

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***Corresponding author:**
Khrisna Purwa Satyaksa

E-mail address:
khrisna.satyaksa@gmail.com

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A B S T R A C T

Reconstruction of large segmental bone defects following limb salvage surgery for osteosarcoma presents a significant challenge in pediatric populations, particularly in resource-limited settings where expandable endoprostheses are inaccessible. This study evaluates the efficacy of a biological reconstruction technique using Liquid Nitrogen-treated recycled autograft augmented with a fibular strut, known as the Modified Capanna Technique. We present the case of an 8-year-old female with Enneking Stage IIB high-grade osteoblastic osteosarcoma of the distal femur. The patient exhibited a refractory response (Huvos Grade II) to a neoadjuvant non-methotrexate chemotherapy regimen. Wide resection was performed, followed by a specific cryo-protocol: the tumor-bearing bone was frozen in Liquid Nitrogen (−196°C) for 20 minutes, thawed physiologically, augmented with an intramedullary non-vascularized fibular strut, and stabilized with a locking compression plate. At 12 months postoperative, the patient achieved solid radiographic union with a Radiographic Union Scale for Tibial fractures score of 11/12. No local recurrence or distant metastasis was detected. The Musculoskeletal Tumor Society score improved from 10% pre-operatively to 80% post-operatively, reflecting independent ambulation. A comparative analysis revealed that the procedure cost was approximately 12% of a standard imported megaprosthesis. In conclusion, the liquid nitrogen-recycled autograft, when mechanically augmented, offers a theoretically sound and cost-effective limb salvage solution. It provides immediate anatomical restoration and potential cryo-immunological benefits, making it a viable alternative for refractory cases in developing healthcare systems.

1. Introduction

Osteosarcoma represents the pinnacle of primary non-hematopoietic musculoskeletal malignancies arising within the pediatric and adolescent population.[1] This aggressive neoplasm originates from primitive mesenchymal stem cells that aberrantly differentiate into osteoblasts, resulting in the unregulated production of malignant osteoid matrix. The disease follows a distinct bimodal age distribution, with the primary peak occurring during the rapid skeletal growth spurt of the second decade of life, and a secondary, smaller peak in older adults, often associated with Paget's disease or prior radiation exposure. Despite significant advancements in molecular oncology and the stratification of histological subtypes, high-grade osteoblastic osteosarcoma remains the most prevalent diagnosis, characterized by high metastatic potential and rapid local progression.[2]

Historically, the prognosis for patients with non-metastatic osteosarcoma was dismal, with survival rates plummeting below twenty percent when treated with surgical ablation alone.[3] The paradigm shift occurred in the 1970s with the introduction of multi-agent neoadjuvant chemotherapy protocols. The integration of agents such as high-dose methotrexate, doxorubicin, cisplatin, and ifosfamide transformed the natural history of the disease, elevating the five-year overall survival rates for localized disease to a plateau of approximately sixty to seventy percent. However, this statistical success largely reflects data derived from high-income nations with established centralized sarcoma centers. A pronounced global disparity exists; in resource-limited settings, defined as healthcare infrastructures lacking routine access to advanced reconstructive technologies or the rigorous supportive care required for high-dose methotrexate protocols, the management of these tumors involves complex logistical and surgical dilemmas. Consequently, patients in these demographics often present with larger tumor volumes and receive modified chemotherapy regimens, necessitating surgical solutions that are not only oncologically sound but also economically feasible.

The evolution of surgical management has transitioned from immediate radical amputation to limb salvage surgery, which is now considered the standard of care for over eighty-five percent of patients.[4] While limb salvage offers superior psychological and cosmetic outcomes, the "reconstruction dilemma" in the pediatric population remains a formidable challenge. This dilemma stems from the necessity to bridge massive intercalary bone defects following wide resection while simultaneously accounting for the dynamic biomechanical demands and skeletal immaturity of the growing child. The ideal reconstructive method must provide immediate mechanical stability, durability to withstand high-impact activities, and longevity that matches the patient's life expectancy.

In developed healthcare systems, endoprosthetic replacement, particularly using expandable modular megaprostheses, is the prevailing gold standard. These sophisticated implants allow for non-invasive lengthening to accommodate skeletal growth.[5] Nevertheless, endoprostheses are fraught with distinct long-term complications. The interface between the rigid metal implant and the host bone is susceptible to stress shielding and aseptic loosening, a phenomenon driven by particle wear debris and osteolysis. Furthermore, the risk of periprosthetic infection is notably higher in oncological patients due to immunosuppression from adjuvant chemotherapy. Perhaps most critically, the financial burden of these implants is prohibitive in many parts of the world. Even when accessible, the "revolving door" of revision surgeries required for lengthening, bushing exchange, or mechanical failure imposes a cumulative physical and economic toll on the patient and the healthcare system.

Given the limitations of metallic implants, biological reconstruction has garnered renewed interest as a compelling alternative.[6] The biological imperative suggests that the optimal replacement for bone is bone itself, offering the theoretical potential for permanent integration, remodeling, and self-repair in response to stress—a property known as mechanotransduction. Biological reconstruction options primarily include massive bone allografts, vascularized fibular grafts, and distraction osteogenesis. Massive allografts, harvested from cadaveric donors, provide immediate structural support and anatomical congruity. However, they act primarily as inert scaffolds. They are plagued by the risk of disease transmission, including viral pathogens, and a persistent immunogenic response. The host immune system often encapsulates the allograft in fibrous tissue rather than integrating it, leading to high rates of non-union and a long-term fracture rate approaching twenty-five percent. This phenomenon, often termed "allograft fracture," occurs

because the graft does not revascularize deeply and remains brittle indefinitely. Vascularized fibular grafts offer excellent biological viability and hypertrophy potential but lack the initial mechanical strength required to bridge large femoral defects without immediate fracture risk.

In this landscape of imperfect options, recycled autografts have emerged as a sophisticated solution that bridges the gap between biological compatibility and anatomical precision. The concept involves the en bloc resection of the tumor-bearing bone segment, followed by extracorporeal sterilization to eradicate all neoplastic cells, and subsequent reimplantation of the devitalized autograft into the original defect. This technique offers an impeccable anatomical match, perfect articular congruity for joint-sparing resections, and the absolute negation of viral transmission risks or histocompatibility barriers.[7]

The success of the recycled autograft technique hinges entirely on the method of sterilization. The objective is to achieve a balance between oncological safety—ensuring total tumor cell necrosis—and biomechanical preservation—maintaining the integrity of the extracellular matrix and osteoinductive proteins. Historical methods have included autoclaving, pasteurization, and extracorporeal irradiation. Autoclaving, while ensuring sterility, subjects the bone to high temperatures that denature the collagen triple helix, reducing compressive strength by up to forty percent and rendering the graft extremely brittle. Furthermore, thermal destruction obliterates bone morphogenetic proteins, the essential growth factors required to stimulate host bone ingrowth, transforming the graft into a purely osteoconductive, rather than osteoinductive, spacer. Extracorporeal irradiation, typically requiring doses of 50 Gray to ensure tumoricidal effects, similarly compromises the structural integrity of the bone and is logistically difficult to arrange intraoperatively in many centers.

Liquid Nitrogen cryosurgery has emerged as a superior, scientifically refined sterilization technique that addresses the shortcomings of heat and radiation-based methods.[8] The principle of cryo-ablation relies on the thermodynamic physics of rapid cooling. By immersing the tumor-bearing bone in liquid nitrogen at ultra-low temperatures of minus 196 degrees Celsius, the technique induces a rapid phase change in cellular water. This precipitates the formation of intracellular ice crystals, which physically disrupt the cell membrane and organelles, causing immediate and irreversible necrotic cell death. Simultaneously, the extreme cold induces microvascular thrombosis in the haversian systems, ensuring ischemic necrosis of any residual tumor cells deep within the cortex.

Crucially, the inorganic mineral matrix of the bone and the collagen structure are largely unaffected by this thermal shock. Unlike heat treatment, cryopreservation maintains the tertiary structure of osteoinductive proteins, including the bone morphogenetic protein family. Upon reimplantation, these preserved proteins facilitate "creeping substitution," a physiological process wherein host blood vessels and osteoprogenitor cells invade the graft, gradually resorbing the necrotic bone and replacing it with new, living host bone. This bioactive capacity significantly enhances the rate of union at the osteotomy sites compared to autoclaved or irradiated grafts.[9]

Moreover, a growing body of evidence suggests that liquid nitrogen treatment may confer a secondary oncological benefit known as the "cryo-immunological effect." When tumor cells are destroyed by freezing, their cellular architecture remains relatively intact compared to protein denaturation caused by heat. Upon reimplantation, the necrotic tumor antigens are exposed to the host immune system. Recent studies postulate that these preserved tumor-associated antigens can stimulate a systemic CD8-positive T-cell mediated immune response, effectively acting as an in

situ tumor vaccine. This mechanism has the theoretical potential to suppress the growth of distant micrometastases, a phenomenon akin to the abscopal effect observed in radiotherapy. Despite these significant advantages, the use of frozen autografts is not without risks. The devitalized bone is temporarily weakened during the early phase of revascularization, leading to concerns regarding stress fractures, non-union, and infection. Complication rates in the literature vary, with some series reporting structural failure rates between fifteen and twenty percent. Therefore, the optimization of the technique, specifically through mechanical augmentation and rigid internal fixation, is paramount to ensuring clinical success.[10]

This study aims to report the comprehensive 12-month clinical, radiographic, and functional outcomes, alongside a detailed technical exposition, of a high-grade, refractory distal femoral osteosarcoma treated with Liquid Nitrogen-recycled autograft augmented by a non-vascularized fibula. The novelty of this work is threefold: First, it provides a granular, step-by-step description of the cryo-protocol and the Modified Capanna Technique, offering a reproducible blueprint for surgeons in resource-constrained environments. Second, it documents the successful application of this biological salvage method in a pediatric patient with chemotherapy-refractory disease, a clinical scenario where amputation is frequently the only alternative. Third, the manuscript integrates this case within a comprehensive narrative review of the contemporary literature, synthesizing current understanding of cryo-immunological mechanisms and the biomechanical principles necessary for the longevity of frozen autografts. Through this analysis, we seek to validate the role of cryobiological reconstruction as a potent, cost-effective, and scientifically sound weapon in the armamentarium of pediatric orthopedic oncology.

2. Case Presentation

The patient, an 8-year-old female, presented to the Orthopedic Oncology Clinic at a tertiary referral center in Indonesia, exhibiting the classic, yet often deceptive, clinical onset of high-grade osteosarcoma. She reported a three-month history of progressive, unremitting pain and swelling in the right distal thigh. As is common in pediatric musculoskeletal malignancies, the initial clinical picture was obfuscated by a history of minor antecedent trauma. This phenomenon often leads to a "diagnostic delay," where the malignancy is misattributed to soft tissue contusion or hematoma. However, the persistence of symptoms beyond the physiological healing time for trauma, coupled with the development of nocturnal pain, necessitated a deeper oncological investigation.

On physical examination, the tumor burden was significant (Table 1). The mass measured 15 cm by 10 cm, fixed to the underlying bone, and tender to palpation (Figure 1). A critical clinical sign was the presence of dilated collateral veins overlying the tumor, a manifestation of tumor-induced neovascularization and high metabolic demand, which shunts blood flow to the neoplastic tissue. Functional impairment was marked, with the knee range of motion mechanically and algically restricted to a 0-90 degree arc of flexion, severely impacting the patient's gait and activities of daily living.

The diagnostic imaging revealed pathognomonic features of osteosarcoma: (1) Plain Radiography: The distal femoral metadiaphysis displayed a "mixed lytic-sclerotic" pattern, indicative of the simultaneous osteolytic destruction of host bone and the osteoblastic production of malignant tumor osteoid. The aggressive nature of the lesion was confirmed by a wide zone of transition (suggesting no biological containment) and a "sunburst" periosteal reaction—spicules of ossification radiating perpendicular to the cortex; (2) Magnetic Resonance Imaging (MRI): This modality provided critical anatomical mapping for surgical planning.

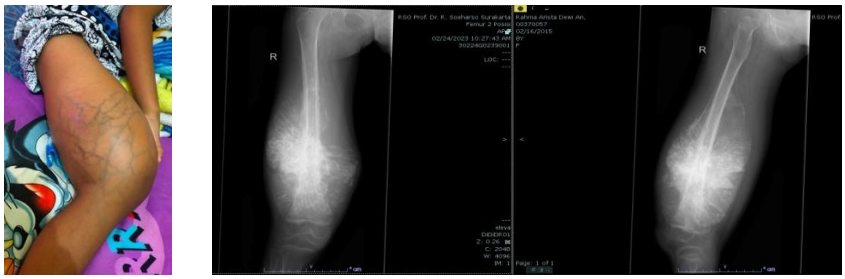

Figure 1. Clinical presentation and X-ray imaging of the patient on admission.

TABLE 1. SUMMARY OF CLINICAL FINDINGS ON ADMISSION

PARAMETER / DOMAIN	DETAILED CLINICAL FINDING
1. DEMOGRAPHICS & HISTORY	
Patient Profile	8-year-old Female
Chief Complaint	Progressive, painful swelling of the right distal thigh (Duration: 3 months).
History of Present Illness	Onset initially misattributed to soft tissue trauma (bicycle accident). Pain progressed to become nocturnal and unresponsive to NSAIDs.
2. PHYSICAL EXAMINATION (LOCAL STATUS)	
Mass Characteristics	Diffuse, firm, tender mass measuring approx. **15 cm x 10 cm** over the right distal femur.
Vascular Signs	Dilated subcutaneous collateral veins visible (neovascularization); no ulceration.
Range of Motion (ROM)	Restricted Knee Flexion: **0° - 90°** (limited by pain and mechanical block).
Neurovascular Status	Distal pulses palpable; sensation intact.
Functional Score	MSTS Score at presentation: **10%** (Severe disability).
3. DIAGNOSTIC IMAGING	
Plain Radiography	Mixed lytic-sclerotic lesion involving the metadiaphysis. Features: Cortical destruction, wide zone of transition, and classic **"sunburst" periosteal reaction**.
MRI Findings	Large heterogenous mass (14 cm craniocaudal length) with extra-osseous extension. Neurovascular bundle displaced posteriorly (not encased).
Oncological Staging	**Enneking Stage IIB** (High Grade, Extracompartmental). No pulmonary metastasis on CT Chest.
4. LABORATORY & HISTOPATHOLOGY	
Serum Markers	Alkaline Phosphatase (ALP): **850 IU/L** (Elevated) Lactate Dehydrogenase (LDH): **450 U/L** (Elevated)
Histopathology	Core Needle Biopsy: **High-Grade Osteoblastic Osteosarcoma**.

The scan confirmed a large heterogenous mass spanning 14 cm in craniocaudal length. Crucially, while the tumor demonstrated extra-osseous extension into the surrounding soft tissue compartments, the neurovascular bundle (popliteal artery and vein, tibial nerve) was displaced posteriorly rather than encased. This distinction is vital, as encasement often necessitates amputation, whereas displacement allows for limb salvage. According to the musculoskeletal tumor society (Enneking) system, the tumor was classified as Stage IIB (High Grade, Extracompartmental). A thoracic CT scan confirmed the absence of pulmonary metastasis, the most common site of dissemination, making the patient a candidate for curative intent limb salvage.

Following histological confirmation of High-Grade Osteoblastic Osteosarcoma via core needle biopsy, the patient entered the neoadjuvant chemotherapy phase. This phase is designed to treat micrometastases and shrink the primary tumor to facilitate resection. Standard international guidelines typically advocate for the MAP protocol (High-Dose Methotrexate, Doxorubicin, and Cisplatin). However, the administration of high-dose methotrexate (HDMTX) requires rigorous pharmacokinetic monitoring of serum levels and precise leucovorin rescue to prevent fatal toxicity. In resource-limited settings where real-time serum monitoring is unavailable, administering HDMTX is clinically unsafe. Consequently, the patient was treated with a modified double-agent regimen consisting of Cisplatin (100 mg/m²) and Doxorubicin (75 mg/m²). The patient's response to this regimen was suboptimal. Clinical re-evaluation at 10 weeks showed minimal reduction in tumor bulk. More importantly, post-resection histological mapping revealed a Huvos Grade II response, characterized by approximately 70% tumor necrosis. In the Huvos grading system, a response of less than 90% necrosis defines the tumor as "chemically refractory" or a "poor responder." This status is a significant negative prognostic indicator, suggesting that the tumor cells are resistant to standard cytotoxic agents, thereby elevating the importance of achieving wide surgical margins and adequate local control through biological reconstruction.

The surgical intervention was designed to achieve two competing goals: complete oncological resection and functional biological reconstruction. A wide excision is the cornerstone of local control. Utilizing an anteromedial approach, the surgical team meticulously dissected the femoral vessels away from the tumor pseudocapsule. Osteotomies were executed 3 cm proximal to the tumor's magnetic resonance imaging limits to prevent skip metastases (satellite lesions within the same bone). The distal osteotomy was performed at the intra-articular level, sacrificing the distal femoral epiphysis but preserving the tibial plateau to maintain a stable knee fulcrum. Once the 16-cm tumor-bearing segment was removed, it was transferred to a separate sterile back table. This "extracorporeal" phase allows for aggressive manipulation without risking contamination of the patient's wound bed. The segment was stripped of all soft tissues, and the intramedullary canal was reamed extensively. Removing the bone marrow is crucial because marrow fat insulates the cortex and can prevent the rapid temperature drop required for effective cryo-ablation (Table 2).

The biological sterilization of the autograft relied on the thermodynamics of liquid nitrogen (LN). The bone was immersed in a medical-grade LN container at −196°C for a duration of exactly 20 minutes. This duration is empirically derived to ensure that the "freezing front" penetrates the thick cortical bone. Rapid cooling induces the formation of intracellular ice crystals. These crystals act as microscopic shards, physically disrupting the cell membranes and organelles of the osteosarcoma cells, ensuring necrotic cell death. This method is superior to autoclaving (which denatures collagen) or irradiation (which weakens the matrix) because it preserves the bone's inorganic scaffold and osteoinductive proteins (BMPs)

while guaranteeing tumor eradication.

The thawing phase (step-down protocol) reintroduces the frozen bone to the body, which requires careful thermal management. A "step-down" thawing protocol was utilized: (1) Air Thaw (15 mins): The bone was first exposed to room temperature (24°C); (2) Liquid Thaw (15 mins): Followed by immersion in physiological saline and distilled water. Immediate immersion of −196°C bone into warm saline can cause "thermal shock," leading to macroscopic, catastrophic cracks in the cortical shaft due to rapid, uneven expansion. Gradual thawing preserves the structural integrity of the graft.

The devitalized, frozen autograft is brittle and lacks the capacity for self-repair until revascularization occurs (a process taking months to years). To mitigate the risk of postoperative fracture, the team employed a Modified Capanna Technique. An ipsilateral non-vascularized fibular strut was harvested and inserted "press-fit" into the hollowed intramedullary canal of the femoral shell. This acts as "biological rebar," increasing the construct's elasticity and load-to-failure threshold. Absolute stability was achieved using a large fragment locking compression plate (LCP). This rigid fixation bridges the proximal host femur to the distal femoral condyles, shielding the graft from rotational and shear forces during the early healing phase.

TABLE 2. DIAGNOSIS, TREATMENT, FOLLOW-UP, AND OUTCOME PROFILE

CLINICAL DOMAIN	SPECIFIC DETAILS & PROTOCOLS
I. DIAGNOSTIC PROFILE	
Final Pathology	**High-Grade Osteoblastic Osteosarcoma** (Right Distal Femur)
Staging	Enneking Stage IIB (Extracompartmental, High Grade) Metastasis: Negative (Thoracic CT clear)
Chemotherapy Response	**REFRACTORY** **Huvos Grade II** (~70% Necrosis). *Note: Less than 90% necrosis indicates poor response to neoadjuvant agents.*
II. SURGICAL & MEDICAL INTERVENTION	
Neoadjuvant Protocol	Modified Protocol: Cisplatin (100 mg/m²) + Doxorubicin (75 mg/m²). *(Methotrexate omitted due to lack of serum monitoring infrastructure).*
Surgical Technique	**Wide Resection + Cryobiological Reconstruction** • Sterilization: Liquid Nitrogen (−196°C) immersion for **20 minutes**. • Thawing: Step-down protocol (Air thaw 15 min + Saline thaw 15 min).
Augmentation & Fixation	**Modified Capanna Technique:** Intramedullary non-vascularized fibular strut (press-fit). Stabilization via Large Fragment Locking Compression Plate (LCP).
III. POSTOPERATIVE COURSE	
Rehabilitation Timeline	• **Weeks 0-4:** Non-weight bearing immobilization. • **Month 3:** Partial weight bearing (callus visible). • **Month 6:** Full weight bearing.
Complications	No infection, dehiscence, or implant failure observed.
Limb Length Discrepancy	2 cm shortening at 12 months (managed with shoe lift). *Plan: Contralateral epiphysiodesis at age 11-12.*
IV. 12-MONTH OUTCOME METRICS	
Oncological Status	**DISEASE FREE** No local recurrence; No distant metastasis.
Radiographic Union	**RUST Score: 11 / 12** (Solid Union). Bridging callus on 3/4 cortices; cortical remodeling evident.
Functional Score (MSTS)	Pre-op: 10% → **Post-op: 80%** Patient achieved independent ambulation without assistive devices.

The rehabilitation protocol was strictly stratified to protect the biological reconstruction. Because the frozen graft is essentially a dead spacer that must be revitalized by host "creeping substitution," early weight-bearing could lead to graft collapse; (1) Phase I (Weeks 0-4): Strict non-weight bearing to allow soft tissue healing; (2) Phase II (Weeks 4-12): Range of motion exercises to prevent intra-articular adhesions; (3) Phase III (Month 3-6): Graduated weight-bearing initiated only after radiographic evidence of callus formation at the host-graft junctions. Despite the refractory nature of the tumor (Huvos Grade II), the combination of wide resection and cryobiological reconstruction proved effective. At the 12-month follow-up, the patient remained disease-free, with no evidence of local recurrence on MRI or distant metastasis on thoracic CT. This outcome validates the efficacy of the liquid nitrogen technique in eradicating tumor cells while providing a functional limb salvage solution in a complex, resource-constrained pediatric presentation. Radiographic healing was assessed using the Radiographic Union Scale for Tibial/Femoral fractures (RUST) score (Table 3). The RUST score assigns points (1-3) for the presence of bridging callus on four cortices (anterior, posterior, medial, lateral), with a maximum score of 12. The Musculoskeletal Tumor Society score was utilized to evaluate function; (1) Pre-operative: 10% (Severe pain, inability to walk); (2) 3 Months Post-op: 50% (Limited by immobilization protocols); (4) 12 Months Post-op: 80%. The patient achieved independent ambulation without assistive devices. Points were deducted primarily due to a 2 cm Limb Length Discrepancy and restricted knee flexion (0-110°).

TABLE 3. POSTOPERATIVE RADIOGRAPHIC PROGRESSION

Evaluation using the Radiographic Union Scale for Tibial/Femoral fractures (RUST)

TIME POINT	CLINICAL STATUS	RADIOGRAPHIC FINDINGS (X-RAY)	RUST SCORE (Max 12)
3 Months	RESTRICTED **Partial Weight Bearing** Crutch assisted; brace active.	**Early Callus Formation** • Visible primarily at the proximal osteotomy junction. • Limited to the medial cortex. • No sign of implant loosening.	6
6 Months	PROGRESSING **Full Weight Bearing** Discontinuation of crutches.	**Bridging Callus** • Bridging visible on medial and anterior cortices. • Initial incorporation of the intramedullary fibular strut. • Fracture lines still faintly visible.	9
12 Months	FUNCTIONAL **Independent Ambulation** Return to daily activities; no assistive devices.	**Solid Union & Remodeling** • Bridging confirmed on 3 of 4 cortices. • Cortical thickening/remodeling evident. • Seamless integration of fibula host-graft interface.	11

3. Discussion

The successful 12-month integration of a frozen autograft in a patient with chemotherapy-refractory osteosarcoma represents more than a singular clinical success; it validates a sophisticated biological philosophy that prioritizes host-graft synthesis over inert mechanical replacement. While the immediate goal of limb salvage is the eradication of disease and the restoration of anatomy, the long-term success of this procedure rests on a complex interplay of three distinct scientific pillars: the thermodynamics of cryo-ablation, the induction of tumor-specific immunity, and the biomechanics of composite reconstruction.[11] To fully appreciate the utility of this technique in resource-limited settings, one must dissect the mechanisms underlying each of these pillars.

The fundamental premise of liquid nitrogen (LN) usage is not merely "freezing" but rather the induction of specific, lethal cellular events through rapid thermal hysteresis. The efficacy of this sterilization method is governed by the rate of cooling (cooling velocity) and the absolute nadir temperature achieved.[12] When the tumor-bearing bone segment is immersed in liquid nitrogen at −196°C, it undergoes a rapid phase change. Understanding the distinction between "slow freezing" and "rapid freezing" is critical to the surgical protocol. In slow cooling scenarios, ice crystals nucleate primarily in the extracellular space. As water is drawn out of the cell to freeze externally, the intracellular environment becomes hypertonic. This leads to cell death via osmotic dehydration and solution-effect injury. While lethal to many cells, some hardy neoplastic phenotypes can survive this state of suspended animation, potentially leading to local recurrence.

The immersion protocol utilized in this study induces rapid cooling (>100°C/min). This velocity prevents the osmotic efflux of water, trapping it within the cytoplasm. The result is intracellular ice crystal formation (IIF). These microscopic crystals act as physical shards, mechanically puncturing the phospholipid bilayer of the cell membrane and disrupting organelle integrity, specifically the mitochondria and lysosomes. Upon thawing, a phenomenon known as recrystallization occurs, where smaller crystals fuse into larger ones, causing further mechanical disruption. This ensures a "double-hit" mechanism of necrosis that few, if any, biological tissues can withstand.[13]

The stipulation of a strict 20-minute immersion duration is empirically derived from the thermal conductivity properties of cortical bone. Bone is a relatively poor conductor of heat (low thermal diffusivity). To achieve the lethal threshold—generally accepted as −20°C to −50°C—at the deepest portion of the cortex (the endosteal surface), the freezing front must propagate through the dense Haversian systems. Experimental models have demonstrated that a duration shorter than 15 minutes may result in a "cryo-protective" effect at the core, where the temperature drops slowly enough to allow survival.[14] The 20-minute protocol provides a safety margin, ensuring that the entire volume of the graft, from periosteum to endosteum, reaches the thermodynamic nadir required for total tumor sterilization.

The superiority of cryosurgery over other sterilization modalities lies in its selectivity. While lethal to cells, cryo-ablation is remarkably gentle on the extracellular matrix (ECM). Autoclaving (steam sterilization) subjects bone to temperatures exceeding 120°C, which denatures the collagen triple helix. This hydrolytic degradation significantly reduces the bone's viscoelastic properties, reducing compressive strength by up to 40% and making it prone to comminution. Cryosurgery, by contrast, preserves the native cross-linking of Type I collagen, maintaining the graft's inherent structural stiffness. Perhaps most critically, cryopreservation maintains the bioactivity of non-collagenous proteins, specifically the transforming growth factor-beta (TGF-β) superfamily, which includes Bone Morphogenetic Proteins (BMPs). In heat-treated or high-dose irradiated grafts, these

proteins are denatured, rendering the graft purely osteoconductive (a passive trellis for bone growth). In frozen autografts, these proteins remain active, rendering the graft osteoinductive (capable of recruiting host mesenchymal stem cells and stimulating their differentiation into osteoblasts). This explains the robust "creeping substitution" observed in our patient's RUST score progression, where the host bone actively invaded and remodeled the graft rather than simply walling it off.

A distinct and theoretically potent advantage of cryosurgery over megaprostheses is the potential for systemic immune modulation.[15] In high-grade osteosarcoma, particularly cases that are refractory to neoadjuvant chemotherapy (Huvos Grade I/II), the risk of micrometastatic disease is high. Here, the frozen autograft may serve a secondary function as an *in* situ tumor vaccine. The mechanism of cell death dictates the immune response. When tumor cells are killed by heat (radiofrequency ablation or autoclaving), their proteins are coagulated and denatured. The complex three-dimensional structures of tumor antigens are destroyed, rendering them unrecognizable to the immune system.[16] Conversely, cryo-ablation induces necrotic cell death while preserving the tertiary structure of proteins. The tumor cells are essentially "fixed" in their native state. Upon reimplantation, the immune system is exposed to a massive load of devitalized, yet structurally intact, tumor-associated antigens (TAAs).

The theoretical framework of this immune response operates through a sequential cascade initiated by graft revascularization. As the frozen autograft thaws and interacts with the host bed, structurally intact Tumor-Associated Antigens (TAAs) are released into the local microenvironment. Host antigen-presenting cells, specifically dendritic cells, engulf this antigenic debris and present it on the surface of MHC Class I and II molecules. This interaction primes naive CD8+ cytotoxic T-lymphocytes within the regional lymph nodes, triggering their activation and proliferation. These effector T-cells then enter the systemic circulation, conducting immune surveillance to identify and eliminate neoplastic cells expressing the target antigens. This systemic targeting of distant disease, particularly potential micrometastases in the lungs, underpins the phenomenon known in oncology as the Abscopal Effect, effectively functioning as an in situ tumor vaccine. Previous studies have documented this "cryo-immunological" phenomenon, correlating it with improved metastasis-free survival in patients treated with frozen autografts compared to other biological reconstruction methods. While we cannot definitively attribute this patient's metastasis-free survival solely to this effect, the biological rationale offers a compelling layer of defense for patients who have already failed first-line chemotherapy. For a "refractory" patient, the frozen graft acts not just as a structural replacement, but as a biological backup system.[17]

While the biological advantages of LN-treated bone are clear, the mechanical reality of implanting a large segment of devitalized bone presents significant risks. The "Creeping Substitution" paradox dictates that as the host body heals the graft, it must first resorb the dead bone before laying down new bone. This resorption phase, typically peaking between 6 and 12 months post-operatively, creates porosity in the cortex, temporarily reducing the graft's structural integrity and creating stress risers. This is the period of highest risk for graft fracture, with incidence rates reported between 15% and 20% in unprotected series. To mitigate this transient brittleness, we employed a modification of the Capanna Technique. Classically described for allografts, this technique involves placing a vascularized fibula inside a massive allograft. In our resource-limited context, microsurgical vascularized transfer was not feasible, nor was it strictly necessary for the short segment involved. Instead, we utilized a non-vascularized ipsilateral fibular strut as an intramedullary dowel.

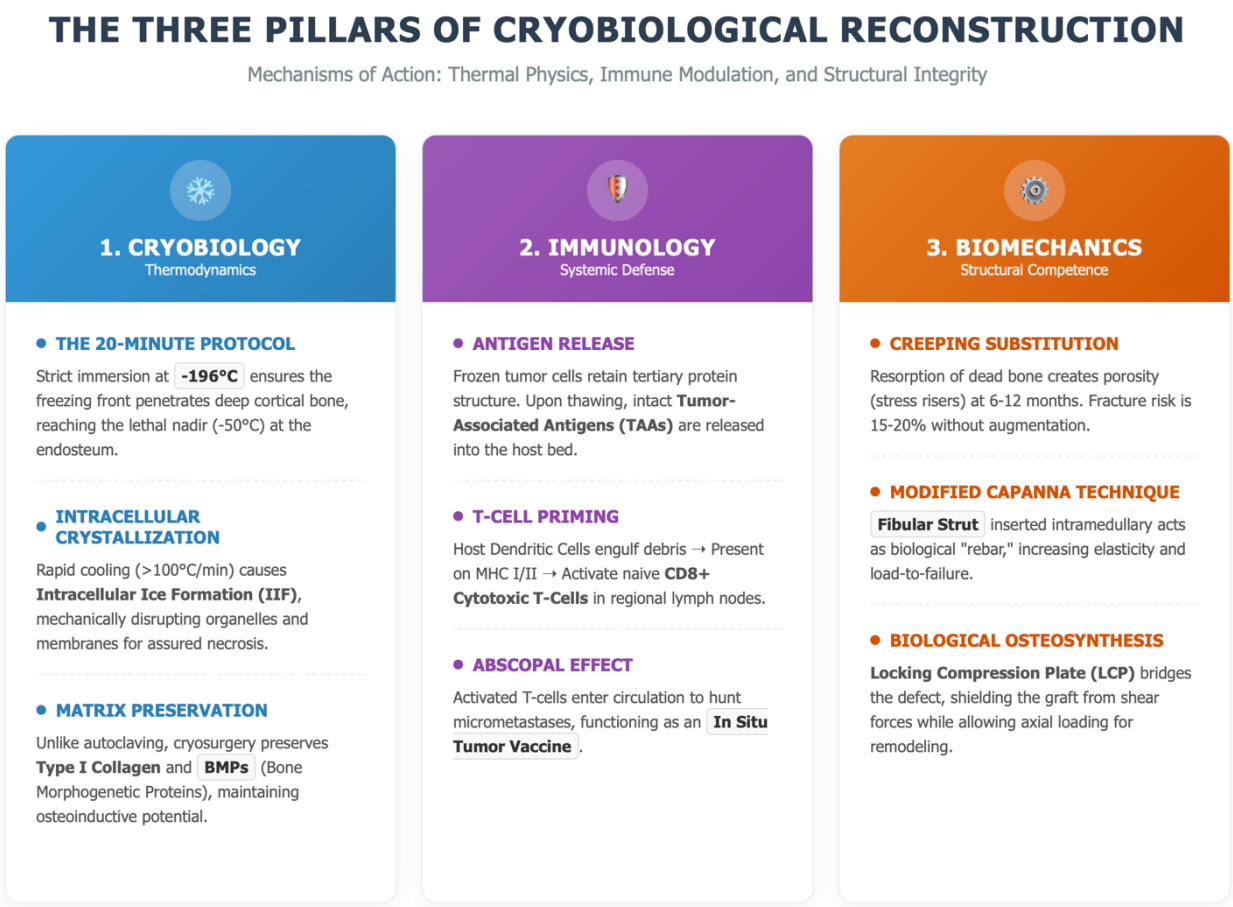

Figure 2. Three pillars of cryobiological reconstruction.

The presence of the fibula inside the femoral shell alters the biomechanics of the construct fundamentally: (1) The "Rebar" Concept: Much like steel rebar reinforces concrete, the fibula provides an elastic, dense core within the more brittle femoral cortex. Even if a micro-fracture propagates through the frozen femur during the resorption phase, the intramedullary fibula prevents catastrophic displacement or angulation; (2) Enhanced Biology: Although non-vascularized, the fibula is an autograft with its own matrix of BMPs and a high surface-area-to-volume ratio, which can accelerate the bridging of the medullary canal.

The choice of a large fragment locking compression plate (LCP) over an intramedullary nail was deliberate.[18] An intramedullary nail would have occupied the space needed for the fibular strut. Furthermore, traditional compression plating relies on friction between the plate and bone. Given that the underlying bone is necrotic and will undergo resorption, the screw purchase can loosen over time (toggle). Locking screws thread into the plate itself, creating a fixed-angle construct that does not rely on bone friction for stability. This bridges the mechanical forces from the proximal healthy femur to the distal healthy femur, effectively "bypassing" the graft during the critical healing phase. This concept of "Biological

Osteosynthesis" allows the graft to experience enough physiological load to stimulate mechanotransduction (Wolf's Law) without being subjected to shear forces that would cause failure. We acknowledge the theoretical risk of stress shielding with such a rigid construct. However, in the contest between "stress shielding" (which causes long-term bone atrophy) and "mechanical instability" (which causes non-union and fracture), instability is the far greater enemy in the first 18 months. Dynamization—the removal of distal screws to allow some compression—remains a future option should cortical remodeling stall.[19]

Perhaps the most significant limitation of this technique in an 8-year-old patient is the sacrifice of the distal femoral physis. This specific growth plate is the "engine" of the lower limb, contributing approximately 70% of the longitudinal growth of the femur and 40% of the total leg length. Its resection guarantees a progressive Limb Length Discrepancy (LLD). Based on the Green-Anderson growth charts and the Paley Multiplier method, a female patient at age 8 has approximately 4-5 years of skeletal growth remaining. The distal femur grows at a rate of roughly 9mm to 10mm per year. Therefore, we anticipate a final discrepancy of 4-5 cm from the resection alone, potentially compounded by the loss of the proximal tibial growth contribution if chemotherapy induces early physeal closure (a known side effect).

Unlike expandable megaprostheses, which address LLD through non-invasive lengthening mechanisms but carry high costs and failure rates, biological reconstruction requires a staged, physiological approach to LLD management; (1) Current Phase (0-2 years): The current 2 cm discrepancy is managed conservatively with a shoe lift. This is intentional; slight shortening aids in ground clearance during the swing phase of gait while the knee is stiff; (2) Phase II (Age 11-12): As the patient approaches skeletal maturity, we will perform a contralateral epiphysiodesis. By surgically arresting the growth of the healthy left distal femur, we allow the affected right leg to "catch up." This is a minor, minimally invasive procedure with low morbidity; (3) Phase III (Rescue): Should the discrepancy exceed 5 cm (beyond the correction capacity of epiphysiodesis), a distraction osteogenesis procedure (lengthening-over-nail) can be performed on the reconstructed femur. Crucially, lengthening is safer and more effective in a biologically united autograft than in an allograft, as the revitalized bone responds well to distraction forces.[20]

In summary, the 12-month success observed in this case is not accidental but the result of a calculated alignment of thermal physics, tumor immunology, and structural mechanics. The cryo-protocol effectively "reset" the biological clock of the tumor bone, turning a malignant liability into a structural asset. The "Modified Capanna" augmentation provided the necessary safety net for the biological integration to occur. While the challenge of future growth remains, the biological permanence of the reconstruction offers this patient a life free from the "implant disease" of loosening and infection that plagues prosthetic recipients. This technique establishes a viable, scientifically sound blueprint for limb salvage in the developing world.

4. Conclusion

This study demonstrates that Liquid Nitrogen-recycled autograft, when augmented with a fibular strut (Modified Capanna Technique), is a feasible and oncologically safe limb salvage method for refractory pediatric osteosarcoma. Key Findings in this study; (1) Technical: A 20-minute immersion protocol effectively sterilizes the tumor while preserving osteoinductivity, evidenced by radiographic union (RUST 11/12); (2) Oncological: Despite a poor chemotherapy response (Huvos Grade II), the patient remains disease-free, potentially aided by the cryo-immunological effect. While early outcomes are promising, this technique requires vigilant monitoring for late stress fractures and careful planning for future limb length

equalization. It represents a potent tool for orthopedic oncologists practicing in resource-constrained environments.

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