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European Association of Urology

Female Urology – Incontinence

Adult Stem Cell Therapy of Female Stress Urinary Incontinence

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Article info

Article history:
Accepted July 12, 2007
Published online ahead of print on July 23, 2007

Keywords:
Incontinence
Myoblasts
Rhabdosphincter
Stem cells
Transurethral ultrasound
Urethra

Abstract

Objectives: To investigate the efficacy of transurethral ultrasound (TUUS)-guided injections of autologous myoblasts and fibroblasts in women with incontinence.

Methods: Between January and June 2005, 20 female patients suffering from stress urinary incontinence (SUI) were included. Skeletal muscle biopsies were taken from the left arm to obtain cultures from autologous fibroblasts and myoblasts. By TUUS guidance the fibroblasts were injected into the urethral submucosa and the myoblasts were injected into the rhabdosphincter. A defined incontinence score, quality-of-life score and urodynamic, electromyographic, and laboratory parameters, as well as morphology and function of urethra and rhabdosphincter were evaluated before and up to 2 yr after therapy.

Results: Eigtheen of 20 patients were cured 1 yr after injection of autologous stem cells and in 2 patients SUI was improved. Two years after therapy 16 of the 18 patients presented as cured, 2 others were improved, and 2 were lost to follow-up. Incontinence and quality-of-life scores were significantly improved postoperatively. The thickness of urethra and rhabdosphincter as well as activity and contractility of the rhabdosphincter were also statistically significantly increased after therapy.

Conclusions: Clinical results demonstrate that SUI can be treated effectively with autologous stem cells. The present data support the conclusion that this therapeutic concept represents an elegant and minimally invasive treatment modality to treat SUI.

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1. Introduction

According to the results of the 3rd International Consultation on Incontinence, approximately 49% of all urinary incontinent women suffer from stress urinary incontinence (SUI), 29% from mixed incontinence, and 22% from urge incontinence [1]. This means that in the majority of incontinent women (78%) the urethral closure complex, that is, the urethra and the rhabdosphincter, would need effective treatment to cure SUI.

The key factors that play a role in SUI include urethral smooth and striated muscle tone and the supportive properties of the urethral mucosa and submucosa, in particular the vascular submucosal layer [2–5]. Standard therapeutic modalities in treatment of SUI (transurethral injections of ''bulking agents'' under endoscopic control, implantation of artificial urinary sphincter systems, suspension and sling procedures, etc) do not treat the pathophysiologic causes of urinary incontinence and often involve the implantation of foreign material. Recent publications support the hypothesis that this represents the major cause that they are ineffective in some patients and may lead to side effects [6–9].

Animal experiments provided the prerequisites for a new kind of therapy: the use of autologous stem cells for reconstruction of the lower urinary tract [10–12]. With this new treatment modality restoration of normal morphology and function of the rhabdosphincter as well as the urethra has become possible.

Therefore, we investigated transurethral ultrasound (TUUS)-guided injections of autologous myoblasts and fibroblasts. Twenty female patients suffering from SUI were treated by means of TUUS-guided injections of autologous myoblasts into the rhabdosphincter and fibroblasts into the urethral submucosa. One- and 2-yr results of these patients are presented in this report.

2. Patients and methods

After approval by the Ministry of Health, 20 female patients suffering from SUI without hypermobility of the urethra and urinary bladder, that is, patients with intrinsic sphincter insufficiency, were treated. The patients were treated between January and June 2005. Written informed consent was provided by all patients. From small skeletal muscle biopsies, two types of muscle-derived autologous cells were isolated, separately grown, and used for therapy:

1. Fibroblasts, which were mixed with 2.5 ml collagen (Contigen®, Bard, USA) as carrier material for the cells before injection so that they did not migrate away from the injection site and injected into the urethral submucosa to treat atrophy of the submucosa.
2. Myogenic stem cells (myoblasts), which were injected directly into the rhabdosphincter to reconstruct defects and improve the function of the muscle (Fig. 1).

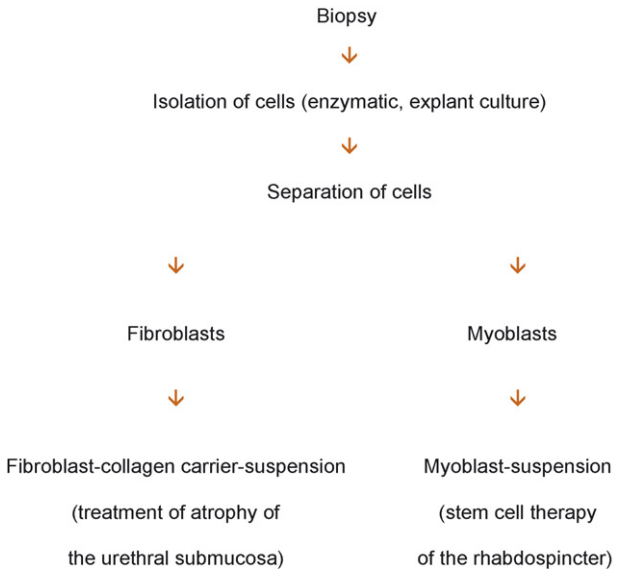

Fig. 1 – Schematic description of stem cell therapy of stress urinary incontinence.

2.1. Outcome (success) criteria

Principally, no generally accepted outcome criteria exist to monitor the success of treatment of incontinence. A vast number of different tests can be used to measure postoperative cure rates. An extensive literature search preceded the selection of four criteria that were evaluated in all patients before and after therapy to quantify postoperative success rates.

1. The Incontinence Score represented the primary parameter for postoperative success of treatment [13]. This parameter is composed of three different criteria and tests (24-h voiding diary, 24-h pad test, patient questionnaire). It allows a good overview of the degree of urinary incontinence before and after therapy as well as the outcome of therapy after injection of the stem cells. The total sum of the score can vary between 6 (totally incontinent) and 0 (completely dry) as shown in Table 1.
2. The Incontinence Quality of Life (I-QOL) Instrument Score reflected quality of life before and after treatment of urinary incontinence [14]. The total sum of all 22 answered questions ranges between 22 (no quality of life at all) and 110 (no restrictions of quality of life). The I-QOL demonstrates the patients' way of life and important behavioral patterns.
3. Sonographic techniques (transurethral ultrasonography, Hitachi 8500) were used to evaluate morphology and function of urethra and rhabdosphincter before and after

Table 1 – The Incontinence Score protocol

1. 24-h miction protocol
   a. No incontinence: 0 points
   b. 1–2 incontinence episodes: 1 point
   c. ≥3 incontinence episodes: 2 points
2. 24-h pad test
   a. Total increase in pad weight ≤8 g: 0 point
   b. Total increase in pad weight 9–20 g: 1 point
   c. Total increase in pad weight ≥21 g: 2 points
3. Patient questionnaire
   a. Patient regards herself as cured: 0 point
   b. Patient regards herself as improved: 1 point
   c. Patient regards herself as not improved: 2 points

therapy [5]. Contractility of the rhabdosphincter was quantified by means of sonographic measurement of defined distances. The distance between the TUUS probe or the urethra and the inner aspects of the rhabdosphincter was measured at rest and during voluntary contraction of the muscle. The difference between these two readings served as parameter for the contractility of the rhabdosphincter [5].

4. Urodynamic tests, including cystometry, pressure-flow studies, urethral pressure profiles, and electromyography (EMG), were performed to investigate the lower urinary tract before and after therapy. Urethral closure pressures were evaluated by means of urethral pressure profiles. A microtransducer system with a diameter of 8F (2.66 mm) was used because the microtransducer technique has been shown to have greater repeatability and reproducibility [15]. Because the profiles show a significant degree of directional (orientation) dependence, all measurements were performed with the transducer face oriented anteriorly (toward the pubic symphysis) and the patients in supine position. Bladder volume was 100 ml in all measurements to further standardize the investigation.

Kinesiologic EMG of the urethral sphincter is an established method for the diagnosis of dysfunction of the rhabdosphincter [15]. Although needle electrodes are less subject to artifacts, surface electrodes measure the activity of the whole rhabdosphincter and are less subject to fluctuations caused by the normal physiologic cycling of motor units. During urodynamic investigation superficial electrodes were placed around the urethral meatus to record the EMG activity in the rhabdosphincter during cystometry and voiding.

2.2. Clinical and laboratory investigations

After the patients consented to the therapy, they first underwent a clinical investigation. Urodynamic and clinical tests, including cystoscopy, pressure-flow studies, lateral x-ray bladder images, and the Q-tip test (mobility $\leq 45^{\circ}$), were performed to investigate the lower urinary tract before therapy to exclude patients with urge incontinence and marked hypermobility of the urethra. Severity of incontinence was quantified by means of the Incontinence Score and quality of life by means of the I-QOL Instrument Score. All patients had performed pelvic floor exercise for at least 6 mo without success prior to the treatment.

2.3. Muscle biopsy

Under local anesthesia, a small incision was made in the left upper arm. A small muscle biopsy (about 0.3 cm^3) was taken and placed in a transport tube containing 10 ml transport medium. The tube was marked with the patient's data and a bar code. The biopsy was then transported to the laboratory.

2.4. Culture and proliferation of cells

Culture of the autologous cells was performed in a ''current good manufactoring practice'' (cGMP) laboratory according to strict standard operating procedures (Fig. 2).

Myoblasts and fibroblasts (two types of muscle-derived autologous cells) were isolated from the muscle biopsies and grown separately.

After about 7 wk the myoblasts were suspended in 1.4 ml Dulbecco modified Eagle medium (DMEM)/F12 with 20% autologous serum, and the fibroblasts in 1 ml DMEM/F12 with 20% autologous serum mixed with 2.5 ml collagen (Contigen®, Bard, USA) as carrier material. The fibroblasts and myoblasts were then filled into separate sterile syringes and transported to the operating room.

2.5. TUUS-guided injection

At the beginning of the cell injection, the TUUS probe (8 Ch, 15–20 MHz) was carefully inserted into the urethra. The urethral wall and the rhabdosphincter were visualized. A specially designed patent-pending injection device was used for precisely adjusted injection of several small portions. First, 15–18 portions (50–100 μl/depot) of the myoblast suspension were injected directly into the omega-shaped rhabdosphincter at two different levels. Then, 25–30 depots (50–100 μl/depot) of the fibroblast/collagen suspension were injected into the submucosa circumferentially at three levels. After implantation of the cells the patients were instructed to perform pelvic floor exercise and transvaginal electrical stimulation for 4 wk postoperatively to support integration of the cells and to improve formation of new muscle tissue.

2.6. Postoperative follow-up

Control visits were conducted at 4 wk, 3 mo, 6 mo, 12 mo, and 24 mo postoperatively. Clinical status, urine and laboratory parameters, Incontinence Score as well as I-QOL Instrument Score were evaluated at every time point. Furthermore, function and morphology of the urethra, the rhabdosphincter, and the urinary bladder were investigated by means of TUUS, urethrocystoscopy, and urodynamic tests at 3 mo, 12 mo, and 24 mo postoperatively.

The preoperative examinations were performed by one urologist (G.P.), the muscle biopsy and cell implanation were performed by another urologist (H.S.), and the postoperative follow-up evaluations were performed by a different urologist (M.M.).

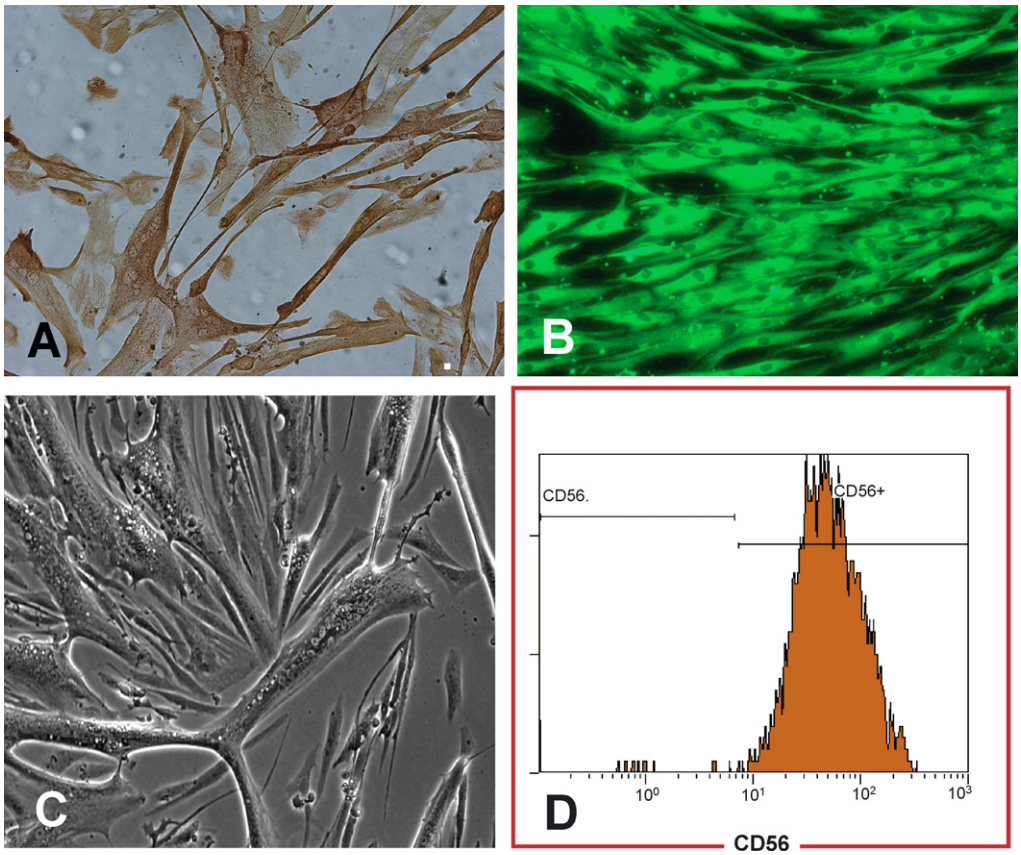

Fig. 2 – Characterization of cells. (A) Immunohistochemical image of autologous human mononucleated myoblasts stained with antidesmin antibodies. (B) Immunofluorescence image of autologous human fibroblasts stained with antivimetin antibodies. (C) Phase contrast microscopic image of multinucleated myotubes that have formed after fusion of mononucleated myoblasts in differentiation medium. With routine phase contrast microscopy of cell samples, fusion of myoblasts could be documented in all myoblast cultures. (D) Fluorescence-activated cell sorting analysis testing the quality of a myoblast cell culture; 97% of the myoblasts are $CD56^+$.

2.7. *Statistical analysis*

The preoperative and postoperative findings were compared using the Wilcoxon test. A $p < 0.05$ was defined as statistically significant.

3. Results

3.1. *Patients*

All 20 patients suffered from SUI without prolapse or hypermobility of the urethra and bladder. They needed to wear 3–5 pads/d. All patients were multipara. Eleven of the patients had undergone abdominal or vaginal hysterectomy with subsequent suspension procedure. The mean age (± standard deviation) of the patients was 49.8 ± 9.2 yr. QOL was severely impaired. Sonographic measurement of contractility of the rhabdosphincter loop preoperatively demonstrated morphologic as well as functional defects of the rhabdosphincter in all 20 patients. All patients had atrophy of the muscle and a reduced contractility. Maximal urethral closure pressures were decreased (Table 2).

3.2. *Injection of stem cells*

Transurethral injection under ultrasound guidance could be performed without problems in all patients. The number of injected fibroblasts ranged between 1.4×10^7 and 6.06×10^7 and the number of injected myoblasts between 1.00×10^7 and 3.00×10^7 cells. The urethral wall and the rhabdosphincter could be visualized exactly. Furthermore, this technique allowed precise application of the myoblasts directly into the rhabdosphincter and of the fibroblasts into the submucosa. The whole injection could be planned, monitored, and documented.

Table 2 – Characteristics of 20 patients treated with autologous cells at baseline and follow-up

	Preoperative	Postoperative follow-up		*p*
		1 yr	2 yr	
No. of patients	20	20	18	
Incontinence Score	6 (6)	0 (0–2)	0 (0–3)	0.001
Quality-of-Life Instrument Score	53 (37–65)	106 (103–109)	104 (102–108)	0.001
Thickness of urethra, mm	4.2 ± 0.9	5.5 ± 1.0	5.3 ± 0.8	0.001
Thickness of rhabdosphincter, mm	1.96 ± 0.5	3.2 ± 0.6	3.4 ± 0.4	0.001
Contractility of rhabdosphincter	0.58 ± 0.3	1.71 ± 0.4	1.75 ± 0.3	0.001
Maximum residual urine, ml	49.2 ± 31.7	12.5 ± 17.0	8.5 ± 9.0	0.001
Maximum urinary flow, ml/s	21.6 ± 5.1	25.2 ± 8.1	23.2 ± 6.1	0.001
Maximum detrusor pressure during flow, cm H_2O	35.4 ± 23.2	30.1 ± 16.7	32.2 ± 15.2	0.001
Maximum bladder capacity, ml	420.1 ± 110	460 ± 90.0	455 ± 120.0	ns
Maximum closure pressure at rest, cm H_2O	27.0 ± 13.3	39.4 ± 14.8	42.2 ± 12.1	0.001
Maximum closure pressure during voluntary contraction of the rhabdosphincter, cm H_2O	37.4 ± 15.5	52.2 ± 17.0	55.3 ± 8.0	0.001
Periurethral EMG at rest, μV	32.0 ± 9.0	45 ± 12.0	47 ± 8.5	0.001
Periurethral EMG during voluntary contraction of the rhabdosphincter, μV	42.1 ± 12.6	56.2 ± 14.2	65.3 ± 18.2	0.001

The numeric variables are presented as mean values and standard deviations, whereas the Incontinence Score and Quality-of-Life Instrument Score (ordinal variables) are presented as median values and range. The statistical differences between preoperative and postoperative are included (Wilcoxon test). Because the *p* value was similar for preoperative to both postoperative comparisons only one value is listed.
ns = not significant.

3.3. *Clinical results*

The muscle biopsy procedure was well acceptabed by the patients, and no infections, inflammations, or postoperative disorders of wound healing were observed.

One year after therapy urinary incontinence was successfully treated in 18 of 20 patients and in 2 patients incontinence was significantly improved. Two years after therapy 16 of 18 patients were cured, 2 patients were improved, and 2 patients were lost to follow-up. None of the cured patients required pads during normal daily life postoperatively. Evaluation of preoperative and postoperative incontinence scores revealed that in these 16 patients urinary incontinence was cured after injection of autologous stem cells (Table 2).

Postoperatively, QOL was statistically significantly improved in all patients. After therapy the patients began to change their lifestyle; all of them said that they were much more satisfied with their social contacts and activities postoperatively (Table 2).

Three months after injection no circumscribed cell depots could be detected in the urethra and the rhabdosphincter. Thicknesses of urethra and rhabdosphincter were increased significantly after surgery. These findings support earlier experimental data that the myoblasts were integrated into the rhabdosphincter. Contractility of the muscle measured by means of TUUS was also significantly improved (Table 2).

TUUS-guided injection of autologous stem cells led to a statistically significant increase in maximal urethral closure pressure. Pressure-flow studies revealed that injection of cells did not cause any obstruction of the lower urinary tract. Micturition was not impaired by the therapy, and all patients could void without residual urine postoperatively. Furthermore, preoperative and postoperative comparison of periurethral EMG activity showed significantly increased EMG activity of the rhabdosphincter postoperatively (Table 2).

In one patient a catheter had to be placed after injection of autologous cells until the first postoperative day. No occurrence of severe postoperative side effects, such as pelvic pain, inflammation, or de novo urgency, occurred. No strictures or scars were detected on transurethral ultrasonography and cystoscopy.

4. Discussion

Urinary incontinence is the most discernible manifestation of several different kinds of injuries and disease processes of the lower urinary tract or the portions of the regulating nervous system. The most common causes of incontinence are detrusor overactivity (neurogenic and idiopathic), generally referred to as urge incontinence, and sphincteric incompetence, resulting in stress incontinence [16].

Factors that contribute to urethral closure include urethral smooth as well as striated muscle tone and the supportive properties of the urethral lamina propria, in particular the vascular submucosal layer. An important contributing factor to stress incon-

tinence, mainly in elderly women with lack of estrogen, may be insufficient mucosal function due to atrophy and reduced vascularization [2]. The striated urethral sphincter, the so-called rhabdosphincter, plays a pivotal role in urinary continence. Sonographic studies have confirmed experimental and clinical data that the rhabdosphincter provides the major muscular portion of the urethral closure mechanism [2–5]. In patients suffering from SUI contractility of the rhabdosphincter is markedly reduced.

The rhabdosphincter is a vertical muscular coat that surrounds the urethra at its ventral and lateral aspects [2–5]. The omega-shaped rhabdosphincter loop pulls the urethra toward the perineal body and compresses the urethra. Injury to the muscle may result from childbirth, maternal injury, or surgical interventions [2]. In addition, age-dependent spontaneous apoptosis leads to a continuous decrease in the density of striated muscle cells in the rhabdosphincter [4]. This loss of rhabdosphincter cells eventually reaches a critical state, which is accompanied by reduced function of the rhabdosphincter and clinically manifest SUI.

The present study demonstrates that SUI can be cured with TUUS-guided injection of autologous adult stem cells. Recent publications have shown that current treatment modalities of urinary incontinence produce good success rates, but the improvement is often short-lived. In addition, standard surgical procedures are associated with side effects and complications [6–9].

Injectables have been shown to provide a minimally invasive but less effective therapy [17]. Due to the limited efficacy and the side effects of standard therapies of urinary incontinence, new minimally invasive and effective treatment modalities are needed. In animal experiments and clinical studies autologous cells (myoblasts and fibroblasts) were injected to treat incontinence and other diseases [10,12–18]. It could be demonstrated that myoblasts display stem cell potential and can differentiate into different types of tissue. Injected autologous myoblasts can be used for reconstruction of striated muscle tissue. So far, no severe side effects have been observed.

One year after therapy 90% of the patients were cured and 10% significantly improved. At the 2-yr control 89% of the patients were cured and 11% improved. All success parameters are substantially improved postoperatively, suggesting that the injected cells have been integrated into the lower urinary tract. The regenerative process stimulated by the myoblasts has led to an increase in the thickness, improved contractility, and increased electrophysiologic activity of the rhabdosphincter. The postoperative ultrasonographic and EMG changes support experimental data that new muscle tissue of the rhabdosphincter is formed and that the cells are integrated into the muscle. The present data show that this new minimally invasive therapy is effective in carefully selected patients.

TUUS was used for preoperative and postoperative evaluation of the urethra and rhabdosphincter. Sonographic measurement of contractility of the rhabdosphincter loop preoperatively demonstrated morphologic as well as functional defects of the rhabdosphincter in all 20 patients. These results are similar to those in earlier publications [5]. Postoperatively, TUUS revealed improved morphology and function of the sphincter complex. The present data strongly support reports that TUUS is mandatory for precise guidance of the injection of the cells [17,18]. In previously performed animal experiments, open surgical, standard endoscopic, or transvaginal ultrasound-guided injections of autologous cells is by far too inaccurate to place the cells exactly in the submucosa and the rhabdosphincter. By way of contrast, TUUS-guided injection proved to be a precise and minimally invasive technique to apply stem cells into the urethra and the rhabdosphincter [12,17,18].

In this report, the 1- and 2-yr results in 20 patients without control group are presented. In another report the efficiency of autologous myoblasts and fibroblasts has been compared with standard endoscopic injections of collagen [18,19]. No data about intraobserver and interobserver variability are available because all injections were performed by the same surgeon. Despite these limitations the present study evaluates and shows the feasibility of using TUUS-guided injection of fibroblasts into the urethral submucosa and myoblasts into the rhabdosphincter to cure and improve SUI in women. If the data from comparative, multicenter, and long-term studies that will be evaluated confirm these findings, this treatment modalitiy has the potential to become a standard treatment of SUI and to represent the first widespread clinical application of autologous adult stem cells in urology [20].

5. Conclusion

Stem cell therapy of SUI offers the possibility to reconstruct (morphologically and functionally) the urethra and the rhabdosphincter. The present data support the conclusion that this new therapeutic concept may represent a promising new and minimally invasive treatment modality.

Conflicts of interest

Dr. M. Fusssenegger is co-owner of IGOR, and Dr. H. Strasser and Dr. R. Marksteiner are founders and co-owners of Innovacell Biotechnologie GmbH. Both companies run certified GMP facilities where the autologous cells were grown. Dr. E. Margreiter, an employee of Innovacell, was responsible for the cell cultures.

Innovacell Biotechnologie GmbH and IGOR provided the cells. In addition, Innovacell Biotechnologie GmbH provided the insurance for the patients. There was no direct payment to the Department of Urology of the Medical University of Innsbruck or any of the authors for performing this study. In addition, neither company had any role in the study design, data collection, data analysis, and data interpretation, because these tasks were solely performed by the authors.

IGOR and Innovacell Biotechnologie had no role in study design, in the collection, analysis, and interpretation of data, in the writing of the report, and in the decision to submit the paper for publication.

All authors provided substantial contributions to the manuscript. The first and all corresponding authors state that they had full access to all the data in the study and had final responsibility for the decision to submit for publication. All authors have read and approved the manuscript.

Acknowledgement

Supported by: FFF-grant 804761, Innovacell grant 001.

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