Autologous Therapies in Dermatology

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ABSTRACT

Autologous therapy is a therapeutic intervention that uses an individual's cells or tissues, which are processed outside the body, and reintroduced into the donor. This emerging field presently represents a mere tip of the iceberg with much knowledge and applications yet to be discovered. It, being free from risks of hypersensitivity reactions and transmission of infectious agents, has been explored in various fields, such as plastic surgery, orthopedics, and dermatology. This review article focuses on various forms of autologous therapies used in dermatology along with their applications and mechanisms of action. (*J Clin Aesthet Dermatol.* 2014;7(12):38–45.)

Autologous therapy is a novel therapeutic intervention that uses an individual's cells or tissues, which are processed outside the body and reintroduced into the donor. Currently, this form of therapy has broad applications in modern medicine, orthopedics, and therapeutic and cosmetic dermatology.

ADVANTAGES OF AUTOLOGOUS THERAPIES

Treatment with allogeneic or synthetic materials, such as bovine collagen, is associated with various adverse effects including allergic reactions, foreign body granuloma formation, and abscess formation. This makes intra-dermal skin testing mandatory prior to their use. These limitations are overcome with autologous therapies, as individual's own cells or tissues are utilized with an added benefit of better clinical results.

VARIOUS FORMS OF AUTOLOGOUS THERAPIES IN DERMATOLOGY

Autologous skin substitutes in dermatology. The first living skin equivalent was developed in 1981 to treat burn victims using sheets of cultured autologous keratinocytes. Although not entirely successful due to skin contracture, scarring, poor epithelialization, and lack of a dermal component, it pioneered a new line of treatment.[1] Since then, several types of nonautologous and autologous skin substitutes have become available with an ever-expanding list of applications (Table 1).

Autologous fat transplants. Autologous fat transplants have been in use for over a century for cosmetic purposes, being first described by Neuber in 1893. Nowadays, it is being used for both therapeutic as well as cosmetic purposes (Table 1). Fat tissue is soft, nonimmunogenic, readily available, and inexpensive. It is a practical option for those patients desiring a more dramatic global change in facial appearance. However, the use of free fat grafting for the treatment of contour abnormalities resulting from breast biopsy or for breast augmentation is generally contraindicated as it can cause palpable nodules and calcifications, situations that may hinder a diagnosis of breast cancer or cause unnecessary intervention.

Surgical procedure: The surgical principle underlying this technique is the atraumatic transfer of fat. The three parts of the surgery are harvesting the graft, transferring the graft, and placing the graft.

Graft harvesting: Autologous fat is harvested with an open-tipped, blunt cannula under tumescent anesthesia. Almost any area can be used as a donar site. The abdomen is easily accessible, and access incisions can be hidden within the umbilicus or in the hair-bearing skin of the pubic area. Common donor sites include periumbilical, lumbar, and trochanteric areas; the thigh; and medial sites of the knee and arm.

Transfer and purification: The aspirate is then transferred through multiple syringes using the tulip connections to 1mL tuberculin syringes. The liquid fraction of the graft is gently washed free of oil, lidocaine, and blood with this transfer. An alternative to gentle hand tipping is use of the centrifuge. The aspirate divides into three layers. The top layer is free oil from ruptured fat cells and is discarded. The bottom layer contains variable

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TABLE 1. Various autologous therapies and their indications in dermatology

AUTOLOGOUS THERAPY			INDICATIONS
Autologous skin substitutes	Cell based	Autologous fat transplant	Facial contouring, lip augmentation, facial rejuvenation, facial scarring including acne scars, penile girth enhancement, vaginal augmentation, atrophic conditions such as Parry-Romberg syndrome, lipodystrophy
		Autologous keratinocytes	Burns
		Autologous dermal papillae cells	Male androgenetic alopecia, female pattern hair loss
		Autologous human fibroblasts	Wrinkles, rhytides, acne scars
	Repigmenting cell-based therapies	Noncultured epidermal cell suspension	Vitiligo, nevus depigmentosum, halo nevus, Piebaldism, post-burn leucoderma, chemical leucoderma
		Cultured pure melanocyte transfer	
		Cultured melanocyte epidermal grafts	
	Repigmenting tissue-based therapies	Mini-grafting	
		Suction blister grafting	
		Split- and full-thickness grafting	
Autologous serum therapy			Allergies, atopic dermatitis, immunodeficiency, vascular diseases, autologous serum skin test (ASST)-positive chronic urticaria
Autologous platelet rich plasma			**Therapeutic indications:** alopecia areata, androgenetic alopecia, improving quality of hair, lipodermatosclerosis **Aesthetic applications:** skin rejuvenation, wrinkles correction, acne scarring, stretch marks, under eye dark circles, striae distensae
Stem cell based autologous therapies	Mesenchymal stem cell based therapies		Wound healing, graft versus host disease, lupus erythematosus, Crohn's disease, cancer therapy
	Hair follicle stem cell based therapies		
Autologous immunotherapy	Dendritic cell based immunotherapy		Malignant melanoma, cutaneous T-cell lymphoma
	T-cell adoptive transfer		Metastatic melanoma
	Autoimplantation therapy		Viral warts

amounts of tumescent fluid and blood and is drained. The middle layer consists of fat cells for grafting. Normal saline or lactated Ringer solution is commonly used for fat suspension.

Placement: Fat is woven into the deep tissues using a small-bore blunt cannula. The level of fat grafting varies with the structure being augmented.

- Kuran's technique for malar, buccal, and mental areas[2]—injection is made in parallel and crossing directions at subcutaneous, intramuscular, and supraperiosteal levels;
- Lip augmentation—grafting near the mucosa;
- Rhytids and prominent nasolabial folds—injections are made parallel to the line of correction.

Serial injections may be performed at three-month intervals with a minimum of three sittings needed in the majority of patients. Complications of this technique include undercorrection, overcorrection, graft necrosis, graft migration, injury to surrounding or underlying structures, infections, and bleeding.

Autologous keratinocytes. Autologous keratinocytes have been used in management of deep, partial-, and full-thickness burns. The study done by Svensjo et al[3] showed faster reepithelialization and better barrier function with keratinocyte transplantation as compared to control wounds. Also, wound reepithelialization and the number of keratinocyte colonies observed in granulation tissue were significantly less in wounds transplanted with noncultured keratinocytes compared to wounds seeded with cultured keratinocytes.[3] It has been seen that 50 to 70 percent take onto debrided wounds with an 86.6 percent survival rate in patients with full-thickness defects three months post-surgery.[4]

Autologous dermal papillae cells. The dermal papilla (DP) comprises fibroblast-like cells that possess an inductive property acquired in the embryo during hair morphogenesis, persisting into adult life. This property is the basis of an emerging cell therapy called follicular cell implantation (FCI) in which dermal papilla cells taken from a few follicles are expanded in culture and then implanted into the skin to induce the formation of many new follicles.[5] This form of therapy has been tried in male androgenetic alopecia and female diffuse alopecia. Many soluble growth factors, especially keratinocyte growth factor (KGF) produced by dermal papillae, act in a paracrine fashion on the overlying epithelial matrix cells to promote hair growth.

Two types of DP cell culture systems that have been tried include one that is based on keratinocytes and another that employs the use of Wnt proteins.[6] The benefits of DP cell culture systems over traditional therapies of alopecia include long-lasting effects (when compared to drug treatment) and not being limited by quantity of donor hair (when compared to hair transplantation).

Autologous human fibroblasts. Autologous human fibroblasts are useful in wrinkles, rhytides, and acne scars. These cells are responsible for the synthesis and secretion of the extracellular environment (collagen, elastin, hyaluronic acid, and glycosaminoglycans) and when injected, can recognize and replenish self-diminished areas in the dermis.[7,8]

Autologous repigmenting therapies (Table 1). There have been some concerns regarding mutagenicity and the possible risk of cancer with the use of cultured melanocyte techniques. 12-tetradecanoylphorbol 13-acetate (TPA), previously used in the culture medium, is a tumor promoter, and concerns about its long-term safety have been raised. Nevertheless, the recent availability of TPA-free and serum- free media has mitigated these concerns to a large extent.

Autologous serum therapy, autologous blood therapy, or autohemotherapy. This involves repeated injections of autologous whole blood or autologous serum. It has been tried over the years in the treatment of a variety of diseases, including chronic inflammation, allergies, atopic dermatitis, immunodeficiency, vascular diseases, osteoarthritis, and autologous serum skin test (ASST)-positive chronic urticaria.[9,10]

While the precise underlying processes in chronic urticaria remain to be identified, possible mechanism includes induction of anti-idiotypes, which inhibit function of disease-producing antibodies.[11] Alternatively, it can skew chronic urticaria Th2 cytokine patterns that promote the induction of urticarial symptoms towards Th1.[12]

Platelet-rich plasma. It is popularly known as vampire facelift in the field of facial rejuvenation. Platelet-rich plasma (PRP) is an autologous preparation of platelets in concentrated plasma. Although the optimal PRP platelet concentration is unclear, it usually contains 300~700 percent enrichment, with platelet concentrations consequently increasing to greater than 1,000,000 platelets/µl.[13,14] The average number of treatments required is two to three separated by a four- to eight-week interval. Its mechanism of action is depicted in Figure 1.

Stem cell based autologous therapies. Stem cells are multipotent cells capable of differentiating into various other cells types. Various sources of autologous stem cells may include bone marrow mesenchymal stem cells (MSC) and cutaneous mesenchymal stem cells. Cutaneous stem cells may be hair follicle stem cells (present in hair follicle bulge),[18] melanocytic stem cells[19] (present in close proximity to keratinocytic stem cells of follicular bulge), and stromal stem cells in subcutaneous tissue.[20]

Role of mesenchymal stem cells in wound repair. Stem cells contribute to wound repair via several of their properties. Firstly, stem cells can produce differentiated skin cells due to their property of self renewal and multipotency.[21] Secondly, the paracrine effect of adult stem cells can promote the progress of wound healing through the release of proangiogenic factors, stimulation of fibroblasts, and collagen synthesis. Thirdly, adult stem cells can modulate the immune and inflammatory responses to promote wound healing. It has been identified that MSC subpopulations can express high levels of the interleukin (IL)-1 receptor antagonist[22] and inhibit inflammatory and immune responses.[23]

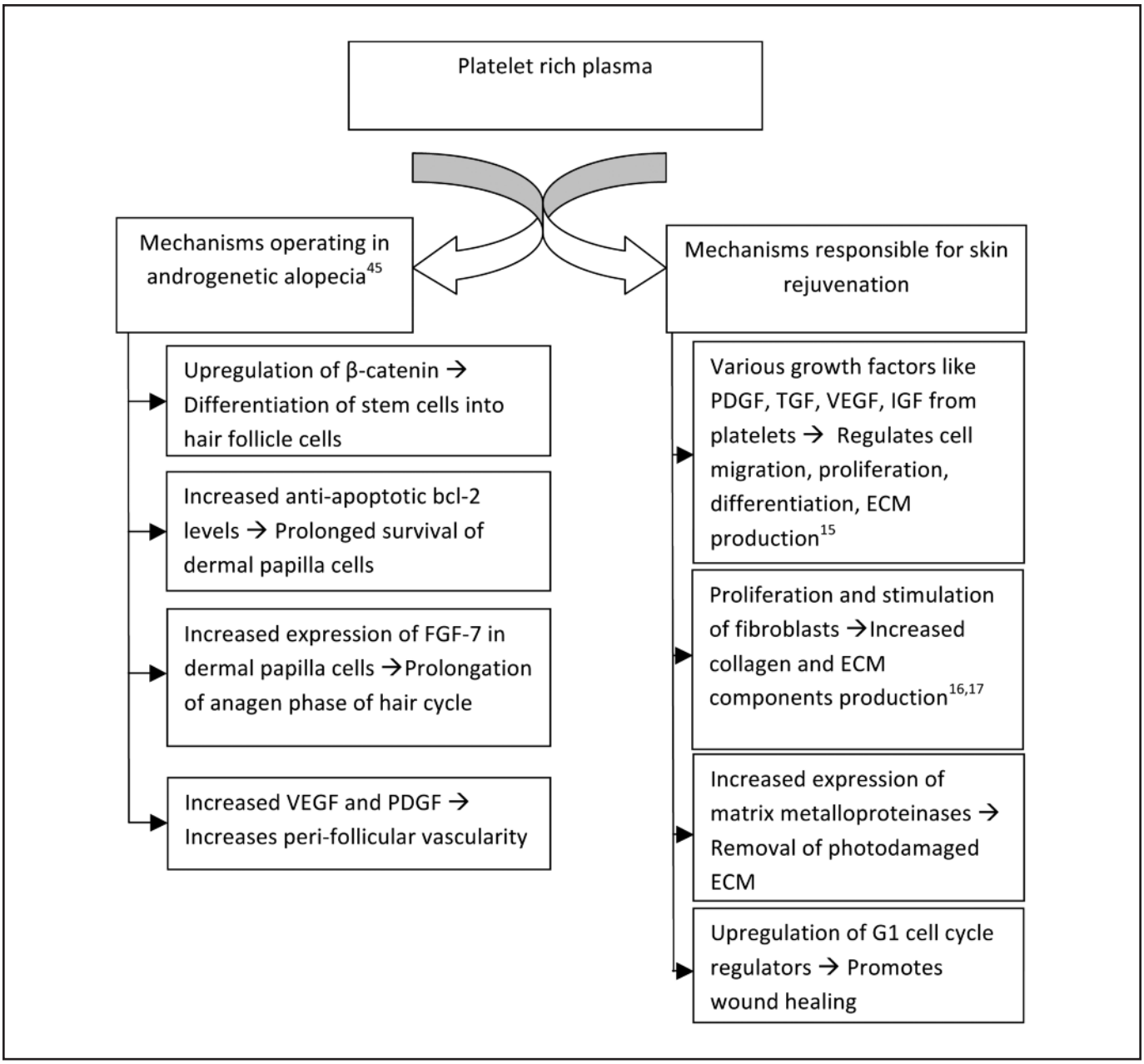

Figure 1. Mechanisms of action of platelet-rich plasma

Role of hair follicle stem cells in alopecia. Hair follicle stem cells reside in hair follicle bulge, a portion in the outer root sheath located in the mid-portion of the hair follicle, which is at the insertion site of the arrector pili muscle. It has been hypothesized that compromising the integrity of the sebaceous gland and/ or bulge is important in the development of alopecia. This has been supported by the fact that in alopecia areata (one of the causes for reversible hair loss), inflammatory infiltrate is centered around the hair bulb, whereas in scarring alopecia, the infiltrate is predominantly focused around the bulge region. Thus, restoration of bulge stem cells may provide a new strategy to control alopecia in the near future. Two main approaches are under investigation—the direct injection of cultured cells or the use of cell secreted factors as a hair growth promoting product. It has been shown that cells from the hair follicle mesenchymal tissue can be cultured and then used to induce new hair follicle formation from epithelial tissue. The injected cells can also migrate to resident hair follicles to increase their size.[24] Alternatively, cells are cultured and the culture supernatant is processed to produce a compound rich in hair growth promoting factors, such as Wnt proteins, for use in treatment. These cell-mediated treatment approaches are still in Phase 1 or 2 trials, but may be available in a few years.

Autologous immunotherapy. *Dendritic cell-based immunotherapy.* Dendritic cells (DCs) are a type of antigen-presenting cells present in skin. Conventional DC-based immunotherapy involves differentiation of blood

TABLE 2. Studies focusing on various autologous therapies in dermatology

AUTOLOGOUS THERAPY	STUDY	REMARKS
Autologous fat transfer	Meier JD, Glasgold RA, Glasgold MJ; 2009[38]	On average, approximately 32% of the injected volume of autologous fat remains at 16 months.
	Cortese A, Savastano G, Felicetta L; 2000[39]	Resorption of 75–98% of the graft, but some good results were obtained with multiple procedures
	Coleman SR; 1995[40]	Patients were monitored clinically with careful photographic controls for 6.5 years.
	Niechajev I, Sevcuk O; 1994[41]	Only partial resorption had occurred 1.5–4.5 years postoperatively. The clinical impression was that 40–50% of the result was maintained long term.
	Ersek RA; 1991[42]	The range of fat resorption was reported at 20–90%
Autologous serum therapy	Pittler MH, Armstrong NC, Cox A, Collier PM, Hart A, Ernst E; 2003[9]	Mean reduction in SASSAD score was 13.5 points (95% confidence interval, CI 6.6–20.4, $p<0.001$) over and above placebo; the corresponding value at the end of treatment was 9.6 (95% CI 4.2–14.9, $p=0.001$). Six patients in the autologous blood therapy group and seven in the placebo group reported minor and transient adverse events.
Platelet rich plasma	Lopez V, Vaya A, Bautista D, Ricart JM; 2013[43]	Significant ($p=0.048$) increase in hair density and borderline increase ($p=0.053$) in hair number in patients with androgenetic alopecia
	Park KY, Kim HK, Kim BJ, Kim MN; 2012[44]	Significant increase in growth rate and hair density was seen without any change in hair thickness in AGA
	Li ZJ, Choi HI, Choi DK, et al; 2012[45]	Near-complete hair regrowth observed at 3 weeks
	Greco J, Brandt R; 2009[46]	9.7% increase in hair shaft diameter at 4 months and 6.1% at 8 months
Dendritic cell-based immunotherapy	Johnson et al; 2009	30% response rate
	Robbins et al; 2011	50% response rate
Autoimplantation therapy for warts	Shivakumar V, Okade R, Rajkumar V; 2009[35]	70% of verruca vulagaris and 80% of palmo-plantar warts showed resolution of warts within 3 months, accounting for a total clearance rate of 73.3%.
	Nischal KC, Sowmya CS, Swaroop MR, Agrawal DP, Basavaraj HB, Sathyanarayana BD; 2012[47]	A total of 20 (74.1%) patients showed a complete clearance of warts within 3 months. Partial clearance was seen in 1 patient. Erythematous nodules developed at the site of implantation in 3 (11.1%) patients. There was relapse in one patient.
	Srivastava PK, Bajaj AK; 2010[36]	Results were evaluated in 53 available patients who turned up for follow-up; 35 patients (66.03%) had complete resolution in 2 months, 12 patients (22.64%) showed partial improvement, whereas six patients (11.32%) had no improvement.

monocytes or CD34+ precursor cells into DCs *in vitro*, followed by tagging with tumor antigens.[25,26]

Another promising strategy is the genetic modification of DCs with tumor antigens. Immature DCs are cotransfected with messenger ribonucleic acid (mRNAs) encoding a tumor antigen, CD40 ligand, CD70, and a constitutively active TLR4.[27] Stimulation of toll-like receptors (TLRs) on DCs causes their maturation and migration to lymph nodes, thereby initiating adaptive immune responses.

A new strategy has been developed to apply protein antigens onto barrier-disrupted skin (epicutaneous immunization), which has been proven to induce long-term cytotoxic T-cell responses.[26] This may lead to a larger entry of immunogenic DCs into the regional lymph nodes and, hence, better immunity compared with vaccination with *in vitro* generated and injected DCs. However, since peptide epitopes are the most common tumor antigen that is incubated with the DCs, the main obstacle is again finding suitable epitopes.

The safety and efficacy of autologous immunotherapy with DCs has been proven in different studies.[28] Toomey et al[29] used dendritic cells pulsed with heat shock protein 70 (Hsp70) and a COX-2 inhibitor in mice. It was shown that Hsp70 induced IL-6 and IL-10 production and suppressed expression of CD40 on DC. This regimen significantly reduced progression of tumors in mice and significantly enhanced survival.

T-cell adoptive transfer. Currently, the most effective immune-based therapy for melanoma is adoptive cell therapy involving the generation of T lymphocytes with anti-tumor activity. The direct targeting of human tumors using autologous tumor infiltrating lymphocytes (TILs) was first demonstrated to mediate tumor regression in 1988, although these results were modest and often not durable.[30]

Techniques of T-cell adoptive transfer:

- Generating TILs—This involves resection of a tumor deposit (generally >1cm, preferably >2cm in diameter) and growth *in vitro* using microcultures in the form of either single cell suspensions or tumor fragments in media containing IL-2. Various limitations of this method include the need for an invasive procedure to procure tumor fragment, tumor location may be inaccessible, and the inability to grow TILs in a subset of patients.
- To overcome above mentioned drawbacks, genes encoding TCRs (T-cell receptors) that recognize tumor antigens can be introduced into a patient's peripheral blood lymphocytes (obtained through aphresis or blood draw) using lentiviral or retroviral vectors.[31]
- However, activity of genetically engineered T lymphocytes is human leukocyte antigen (HLA) restricted. This problem was overcome by the use of chimeric antigen receptors (CAR). A CAR results from the fusion of the intramolecular signaling domain of a TCR (e.g., CD 28) with the extracellular antigen-binding domain that recognizes the tumor antigens. So, these CAR- transduced cells have the combined specificity of an antibody and cytotoxicity of T lymphocytes.

When these TILs are infused into patients along with IL-2 and reduced-intensity chemotherapy to temporarily knock down the patient's circulating immune cells, TIL can mediate tumor responses in up to 70 percent of patients, with a significant portion of these being durable complete responses (defined as the disappearance of all target lesions).[32]

A study done by Motohashi et al has shown a 51-percent objective response rate, with tumors shrinking to undetectable size in some patients.[33,34]

Autoimplantation therapy of warts. This has been tried by autoimplantation of the wart tissue into the uninvolved skin, injecting the suspension of the crushed wart into the muscle or skin.[35,36] The possible mechanism is supposed to be the stimulation of autoimmune process by the injected wart tissue. A significant increase in both antibody and delayed hypersensitivity response are noted after intradermal testing with human papillomavirus antigens and wart treatment.[37] The procedure, as described by Shivakumar et al[35] uses a well-developed verrucous papule as a donor wart. Under local anesthesia and strict aseptic precautions, a full-depth nick is made up to the subcutis level with an 18-gauge needle and a chunk of wart tissue is removed and placed on a sterile swab. An area on the flexor aspect of the left forearm around two inches below the antecubital crease is chosen as a recipient site for autoimplantation. Under local anaesthesia, a pocket is created in the subcutaneous tissue with to and fro motions of the needle, where the harvested tissue is gently introduced and secured tightly with a small micropore dressing or band-aid plaster.

A brief innumeration of various studies focusing on autologous therapies in dermatology is depicted in Table 2.

DRAWBACKS OF AUTOLOGOUS THERAPIES

The major drawbacks of autologous therapies include the need for a highly sophisticated set-up, well-trained dermatologists, and the high cost of treatment, especially in developing countries. Additionally, as processing and preparation of cells and tissues require some time, there is a significant lag before the commencement of a patient's treatment in some procedures.

CONCLUSION

Autologous therapies show promise in dermatology with the ability to overcome some of the hurdles of traditional therapies. In the near future, it is likely that autologous therapies may become a useful and often employed tool in the treatment armamentarium of aesthetic and therapeutic dermatology.

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