EXAMPLE PROJECT PROPOSAL

Background/Significance: Keloids are common fibrotic skin tumors that appear firm and rubbery. Although a keloid is classified as a benign dermal lesion, it mimics certain malignant cell characteristics such as invasion and hyper-proliferation (Bailey et al. 2007; Mari et al. 2015). Keloids can occur in predisposed individuals upon loss of skin integrity following any injury such as due to scratches, body piercings, surgical incisions, insect bites, or spontaneously after allergic reactions (Bailey et al. 2007; Hochman et al. 2015; Mari et al. 2015). They can be pruritic, painful and can become ulcerated or infected, causing great discomfort and adversely affect the patient's quality of life. Keloids form due to aberrant wound healing leading to excessive dermal fibroblast proliferation at the injury site and deposition of types I and III collagen (Nakaoka, Miyauchi, and Miki 1995; Murray, Pollack, and Pinnell 1981; Teofoli et al. 1999; Butler, Longaker, and Yang 2008; Bran et al. 2009). During normal wound healing, homeostasis is maintained between the collagen production and degradation; however, in keloids, this balance is lost, leading to abnormal scarring. The incidence of keloids and their severity is much higher among people of African descent (~15%) and people of color compared to their white counterparts (less than 0.1%), suggesting contributions from genetic and epigenetic factors (Al-Attar et al. 2006; Andrews et al. 2016; Barrett 1973; Bloom 1956; Gauglitz et al. 2011; Glass 2017; Halim et al. 2012; He et al. 2017; Marneros et al. 2001; Smith et al. 2008). However, the genetic alterations and epigenetic mechanisms involved in keloid formation are unknown. Studies suggest epigenetic factors such as DNA methylation (Jones et al. 2015) and post-translational modifications (PTMs) on histone proteins (that package genomic DNA and thereby modulate all DNA functions) are involved in keloid pathogenesis (Son 2022a).

Given that keloids primarily affect people of color, they remain understudied compared to other dermatologic disorders and are thus one of the most challenging dermatological conditions to treat. Since the 1960s, intralesional corticosteroid (or simply "steroid") injections have been used widely for keloid therapy (Jalali and Bayat 2007). Steroids soften and flatten keloids by diminishing collagen synthesis and inhibiting keloid fibroblast proliferation (Boyadjiev, Popchristova, and Mazgalova 1995; Reish and Eriksson 2008). However, recent studies have demonstrated that only ~34% of keloid patients benefit from steroid therapy, while ~49% see no benefits and ~17% of patients exhibit a worsening of their keloids following steroid therapy (Tirgan 2013; Tirgan 2017; Ud-Din et al. 2013). Hence, in most cases, keloid treatment involves surgical removal. Unfortunately, in almost 100% of the cases of keloid removal surgery without any adjuvant therapy, the keloid reappears, making it difficult to provide long-term relief (Arno et al. 2014; Sclafani et al. 1996; Shaffer, Taylor, and Cook-Bolden 2002). Adjuvant post-surgical therapies including intralesional corticosteroids (Berman and Bieley 1996; Jung et al. 2009; Kiil 1977; Ledon et al. 2013; Ogawa 2010; Shin et al. 2016; Shons and Press 1983), radiation therapy (Emad et al. 2010; Flickinger 2011; Gauglitz 2013; Kim and Lee 2012; Lee and Park 2015; Ogawa et al. 2013; Ragoowansi et al. 2003; Son et al. 2020) and other adjuvants (Wilson 2013) can significantly reduce the risk of recurrence following surgery. However, currently there is no gold standard or approved therapy for keloids and they are treated by physicians largely empirically, leading to highly variable outcomes.

A long-term goal of the host lab is to use a comprehensive set of genomic tools to identify the molecular mechanisms of keloid formation and use this information to establish an effective standard for the clinical management of keloids. As a first step towards this goal, the host lab has just completed DNA methylation sequencing (methyl-seq) and RNA sequencing (RNA-seq) analyses, including microRNA sequencing (miRNA-seq), to characterize the DNA methylome and transcriptome of patient-derived normal and keloid fibroblasts (before and after steroid treatment) to understand the molecular pathways involved in keloid formation and their response to steroid therapy (Son 2022a; Son 2022b). These data are already providing great insight into the transcriptomic and DNA methylation changes that drive the molecular pathways involved in keloid formation and response to therapy. However, genomic and epigenomic profiling of keloids have not been attempted so far, and so our understanding of the upstream genomic/epigenomic changes that are likely driving the observed transcriptomic changes in keloids is incomplete.

Hypothesis: My central hypothesis is that the genome and/or the epigenome of keloid cells differ from normal skin cells in their genomic alterations and epigenetic modifications that determine keloid formation and response to therapy. This hypothesis has been formulated based on preliminary data from the host lab's published (Son et al. 2020) and unpublished work (Son 2022a; Son 2022b), and recent literature reviews, which strongly suggest that susceptibility to keloids and response to steroid therapy are likely to be determined genetically and/or epigenetically. The overall objective of this study is to analyze genetic and epigenetic alterations contributing to keloid pathogenesis, especially among individuals of African descent. This information will be crucial for developing improved therapeutic options for keloids. Using patient derived normal skin and keloid dermal fibroblasts, I plan to test the central hypothesis through the following two specific aims:

Aim 1: ***To determine the genomic changes in keloids using whole-genome sequencing (WGS)***. I propose to identify genetic alterations such as point mutations, single nucleotide variations (SNVs), structural variations (SVs) including insertions/deletions (INDELs), inversions, translocations and copy number variations (CNVs) that may be involved in determining susceptibility to keloid disease, or response to therapy.

Aim 2: ***To establish the epigenetic determinants of keloid formation by performing epigenomic profiling of multiple histone modifications.*** For this, I will use the recently developed "Cut & Tag" technology (Kaya-Okur et al, 2019, 2020) to determine patterns of 8 different activating and repressive histone PTMs.

Rationale: Despite keloids being common, currently there are no comprehensive studies using modern genomic sequencing techniques. In the past two decades, only studies on transcriptomic profiling of keloids in response to either steroid treatment (Smith et al. 2008) or radiation (Tosa et al. 2005) using the now obsolete microarray technology have been performed and have provided limited datasets. Although there have been some recent next-generation sequencing (NGS) based transcriptomic analysis of keloids (Jumper et al. 2017; Lee et al. 2019; Son et al. 2020), there are still no data on the genomic and epigenomic determinants of keloids. Additionally, the published studies on genomic analysis of keloids have only identified a few SNVs that are limited to Asian populations (Chung et al. 2011; Fujita et al. 2019; Nakashima et al. 2010; Ogawa et al. 2014; Zhu et al. 2013). Since race may contribute to a higher incidence or greater severity of keloids, applying the findings from one racial group to another such as African Americans is very challenging. Finally, although genetic predisposition may be critical in keloid pathogenesis, several studies have suggested that environmental factors that alter epigenetic marks may also trigger abnormal gene expression in genetically susceptible individuals during the wound healing process (Butler, Longaker, and Yang 2008; Chevray and Manson 2004; Niessen et al. 1999; Phan et al. 2003), although these epigenetic marks are not known. Not surprisingly, these studies have not resulted in any significant clinical advances in over a decade, suggesting that additional factors and pathways that play key roles in keloid formation and response to therapy are yet to be identified. Hence, the application of unbiased genomic sequencing-based approaches to keloids from people of different skin color to understand their genomic and epigenomic differences will be crucial in identifying the molecular mechanisms involved in keloid formation, as well as developing new therapies.

Innovation: The proposed research is innovative because it will be the first comprehensive set of genomic and epigenomic analyses of keloids primarily from African Americans. This will help improve our understanding of keloid biology, establish the molecular determinants of effective therapy, and uncover additional molecular pathways that can be targeted for potential new treatments for keloids in the future.

Preliminary Studies: There are no approved standard keloid therapies and clinical management remains suboptimal, resulting in frequent keloid recurrence. Since keloids are known to occur only in humans and appropriate animal models are lacking, to address this issue, the host lab has been using keloid-derived primary fibroblasts and tissue explants cultured *in vitro* to assess the effectiveness of adjunctive post-excision keloid treatments. So far, their results have shown that a single dose of superficial (i.e., skin deep) radiation following surgical removal of keloids is sufficient to block recurrence (Son et al. 2020) . Additionally, steroid treatment alone resulted in either a modest reduction in proliferation (50% of samples, steroid-sensitive), no significant effects on proliferation (40%, steroid insensitive), or hyperproliferation of some keloid samples (10%) *in vitro*, similar to what is observed in keloid patients (Tirgan 2013; Tirgan 2017). Remarkably, radiation was also effective in eliminating steroid-resistant keloid fibroblasts. Finally, in addition to novel therapeutic targets and a few biomarkers for steroid resistance, the recent transcriptomic and DNA methylome analysis of keloid samples completed by the host lab also suggest that the differential transcriptional and DNA methylation patterns that determine the responses of keloids to steroid therapy are likely to be encoded genetically or epigenetically (Son 2022a; Son 2022b), although these mechanisms have not been studied so far.

Approach: Here, I propose to greatly expand the previous studies from the host lab by identifying the genetic and epigenetic drivers of the transcriptional changes observed previously in keloids (Son 2022a; Son 2022b). For this, I will use the same set of patient derived keloid and normal skin samples that were obtained by the host lab following FSU IRB approval for their transcriptomic/DNA methylome profiling, and include both steroid responsive and resistant keloids. I will perform WGS to identify genetic alterations, while Cut & Tag technology will be carried out to determine the genome wide patterns of specific histone modifications. Cells cultured from a total of 8 samples (five-keloid tissue samples derived from 3 African Americans, 1 Caucasian and 1 Hispanic patient respectively, as well as 3 race-matched normal skin tissue samples), will be used for the experiments in the two proposed Aims. Additional samples may be included as they become available, especially if sequencing costs drop further at the time the experiments are actually being performed.

***Aim 1 - Methods*.** To uncover any genetic alterations that may contribute to keloid pathogenesis and response to steroid therapy, WGS will be performed on the 8 samples listed above. For this, DNA will be extracted and sequencing libraries will be prepared targeting a 350bp insert size. 150bp paired-end WGS will be performed on the Illumina NovaSeq 6000 sequencer available at the FSU College of Medicine. We aim to obtain a 40X coverage of the genome, which is sufficient for obtaining information on almost all SNVs, SVs and CNVs. Reads will be aligned to the latest reference human genome using the Burrows-Wheeler Aligner, flagged for duplication and recalibrated using the Genome Analysis Toolkit. For calling the potential somatic SNVs and small INDELs, we will use MutTeck and SvABA respectively. Somatic mutations will be analyzed using ANNOVAR. We will measure CNVs using Patchwork. We will also perform other analyses using packages such as Meerkat and SvABA to predict somatic SVs and their breakpoints.

***Aim 2 - Methods*.** Epigenetic factors such as histone PTMs may contribute to keloid formation and response to therapy (Son 2022a). Given the vast array of epigenetic modifications, we will focus only on eight site-specific histone modifications involving trimethylation (me3) and acetylation (ac) of histones H3 and H4: H3K4me3 and H3K36me3 (both gene activating methylation marks); H3K27me3 and H3K9me3 (both repressive methylation marks); H3K27ac, H3K9ac, H3K14ac and H4K8ac (all generally activating acetylation marks). Together these modifications would provide a fairly comprehensive coverage of the most important histone modifications that are known to impact transcription. Highly specific, Cut & Tag validated monoclonal antibodies are already available in the lab for all these histone modifications. For Cut & Tag, we will use reagents from a commercial vendor (Epicypher) and follow the protocol established by the Henikoff lab (Kaya-Okur et al. 2020). Briefly, patient-derived keloid or normal skin fibroblasts will be collected from the samples listed above. They will be processed using Cut & Tag's novel protein A fused to the Tn5 transposase enzyme that is conjugated to NGS adapters. After tagmentation and DNA purification, 50bp single-end sequencing will be performed to achieve a sequencing depth of ~5 million reads per sample. Reads will be aligned to the human genome (hg38) using Bowtie2. Duplicates will be removed using Picard. Macs2 will be used for peak calling. Our data will also be compared to epigenomic data from normal and diseased cells publicly available through the ENCODE project.

Expected results, potential problems, and alternative approaches: Given the host lab's recent work on transcriptomic and DNA methylome profiling of keloid cells (Son 2022a; Son 2022b), we do not expect technical challenges with the proposed NGS experiments. WGS data should reveal any genetic alterations that are specific to keloid samples and the most interesting of these will be confirmed using PCR based targeted sequencing, followed by functional assays to further study the role of such alterations in keloid biology. Cut & Tag should reveal the location of any differential histone modifications between keloid and normal cells. Since Cut & Tag is new to the host lab, if it fails for any reason, we will perform traditional chromatin immunoprecipitation sequencing (ChIP-seq) to explore changes in epigenetic histone modifications between normal skin and keloid cells. The locations of the genomic alterations in keloids uncovered by WGS, as well as the epigenomic differences determined by Cut & Tag will then be correlated with the genes exhibiting altered expression or methylation patterns in keloid cells that were previously determined by the host lab using RNA-seq, miRNA-seq and methyl-seq data (Son 2022a; Son 2022b). This will provide mechanistic insights into how the upstream genomic or epigenomic alterations in keloids maybe be driving the downstream gene expression changes, which in turn drive keloid formation and determine their response to therapy. Such data may reveal novel molecular targets for keloid therapeutics, as well as keloid biomarkers that can be utilized to screen and counsel individuals susceptible to keloid formation on ways to mitigate keloid risk following surgeries, and to predict the response of keloid patients to available therapies, as has been done recently for steroid therapy of keloids (Son 2022b). Comparing our keloid epigenomic data to the ENCODE data may shed light on its propensity to affect people of color. Overall, we expect that the WGS and Cut & Tag datasets will add significantly to the list of novel therapeutic targets and potential diagnostic markers uncovered previously by the host lab. Together, these will form the most comprehensive set of genomic data on keloids to date, especially for African American patients, that will serve as a valuable resource for the entire research community, thereby accelerating the pace of keloid research.

Project Timeline: During the ***first six months***, patient-derived normal and keloid fibroblasts will be cultured and harvested for WGS and Cut & Tag. I will perform Cut & Tag assays, isolate DNA, prepare sequencing libraries, run quality control assays on them and then sequence them. I will also learn the computational and bioinformatics data analysis pipelines during this period to get ready for handling the large volume of sequencing data. In the ***next nine months***, I will analyze all the sequencing data to determine genetic and epigenetic differences between keloids and normal skin in steroid-sensitive and steroid-resistant samples among different ethnic groups, and perform statistical analyses. In the ***final six months***, I will prepare manuscripts for publication, apply for independent faculty positions, which will likely also include writing grant proposals for my independent career.

References

Al-Attar, A., S. Mess, J. M. Thomassen, C. L. Kauffman, and S. P. Davison. 2006. 'Keloid pathogenesis and treatment', *Plast Reconstr Surg*, 117: 286-300.

Andrews, J. P., J. Marttala, E. Macarak, J. Rosenbloom, and J. Uitto. 2016. 'Keloids: The paradigm of skin fibrosis - Pathomechanisms and treatment', *Matrix Biol*, 51: 37-46.

Arno, A. I., G. G. Gauglitz, J. P. Barret, and M. G. Jeschke. 2014. 'Up-to-date approach to manage keloids and hypertrophic scars: a useful guide', *Burns*, 40: 1255-66.

Bailey, J. N., A. E. Waite, W. J. Clayton, and M. H. Rustin. 2007. 'Application of topical mitomycin C to the base of shave-removed keloid scars to prevent their recurrence', *Br J Dermatol*, 156: 682-6.

Barrett, J. 1973. 'Keloid In: Bergsma D, editor', *Birth Defect Compendium. Baltimore: Williams and Wilkens Company*: 553.

Berman, B., and H. C. Bieley. 1996. 'Adjunct therapies to surgical management of keloids', *Dermatol Surg*, 22: 126-30.

Bloom, D. 1956. 'Heredity of keloids; review of the literature and report of a family with multiple keloids in five generations', *N Y State J Med*, 56: 511-9.

Boyadjiev, C., E. Popchristova, and J. Mazgalova. 1995. 'Histomorphologic changes in keloids treated with Kenacort', *J Trauma*, 38: 299-302.

Bran, G. M., U. R. Goessler, K. Hormann, F. Riedel, and H. Sadick. 2009. 'Keloids: current concepts of pathogenesis (review)', *Int J Mol Med*, 24: 283-93.

Butler, P. D., M. T. Longaker, and G. P. Yang. 2008. 'Current progress in keloid research and treatment', *J Am Coll Surg*, 206: 731-41.

Chevray, P. M., and P. N. Manson. 2004. 'Keloid scars are formed by polyclonal fibroblasts', *Ann Plast Surg*, 52: 605-8.

Chung, S., M. Nakashima, H. Zembutsu, and Y. Nakamura. 2011. 'Possible involvement of NEDD4 in keloid formation; its critical role in fibroblast proliferation and collagen production', *Proc Jpn Acad Ser B Phys Biol Sci*, 87: 563-73.

Emad, M., S. Omidvari, L. Dastgheib, A. Mortazavi, and H. Ghaem. 2010. 'Surgical excision and immediate postoperative radiotherapy versus cryotherapy and intralesional steroids in the management of keloids: a prospective clinical trial', *Med Princ Pract*, 19: 402-5.

Flickinger, J. C. 2011. 'A radiobiological analysis of multicenter data for postoperative keloid radiotherapy', *Int J Radiat Oncol Biol Phys*, 79: 1164-70.

Fujita, M., Y. Yamamoto, J. J. Jiang, T. Atsumi, Y. Tanaka, T. Ohki, N. Murao, E. Funayama, T. Hayashi, M. Osawa, T. Maeda, D. Kamimura, and M. Murakami. 2019. 'NEDD4 Is Involved in Inflammation Development during Keloid Formation', *J Invest Dermatol*, 139: 333-41.

Gauglitz, G. G. 2013. 'Management of keloids and hypertrophic scars: current and emerging options', *Clin Cosmet Investig Dermatol*, 6: 103-14.

Gauglitz, G. G., H. C. Korting, T. Pavicic, T. Ruzicka, and M. G. Jeschke. 2011. 'Hypertrophic scarring and keloids: pathomechanisms and current and emerging treatment strategies', *Mol Med*, 17: 113-25.

Glass, D. A., 2nd. 2017. 'Current Understanding of the Genetic Causes of Keloid Formation', *J Investig Dermatol Symp Proc*, 18: S50-s53.

Halim, A. S., A. Emami, I. Salahshourifar, and T. P. Kannan. 2012. 'Keloid scarring: understanding the genetic basis, advances, and prospects', *Arch Plast Surg*, 39: 184-9.

He, Y., Z. Deng, M. Alghamdi, L. Lu, M. W. Fear, and L. He. 2017. 'From genetics to epigenetics: new insights into keloid scarring', *Cell Prolif*, 50.

Hochman, B., F. C. Isoldi, F. Furtado, and L. M. Ferreira. 2015. 'New approach to the understanding of keloid: psychoneuroimmune-endocrine aspects', *Clin Cosmet Investig Dermatol*, 8: 67-73.

Jalali, M., and A. Bayat. 2007. 'Current use of steroids in management of abnormal raised skin scars', *Surgeon*, 5: 175-80.

Jones, L. R., W. Young, G. Divine, I. Datta, K. M. Chen, D. Ozog, and M. J. Worsham. 2015. 'Genome-Wide Scan for Methylation Profiles in Keloids', *Dis Markers*, 2015: 943176.

Jumper, N., T. Hodgkinson, R. Paus, and A. Bayat. 2017. 'Site-specific gene expression profiling as a novel strategy for unravelling keloid disease pathobiology', *PLoS One*, 12: e0172955.

Jung, J. Y., M. R. Roh, Y. S. Kwon, and K. Y. Chung. 2009. 'Surgery and perioperative intralesional corticosteroid injection for treating earlobe keloids: a korean experience', *Ann Dermatol*, 21: 221-5.

Kaya-Okur, H.S., Wu, S.J., Codomo, C.A., Pledger, E.S., Bryson, T.D., Henikoff, J.G., Ahmad, K. and Henikoff, S., 2019. 'CUT&Tag for efficient epigenomic profiling of small samples and single cells'. *Nature communications*, *10*: 1-10.

Kaya-Okur, H. S., D. H. Janssens, J. G. Henikoff, K. Ahmad, and S. Henikoff. 2020. 'Efficient low-cost chromatin profiling with CUT&Tag', *Nat Protoc*, 15: 3264-83.

Kiil, J. 1977. 'Keloids treated with topical injections of triamcinolone acetonide (kenalog). Immediate and long-term results', *Scand J Plast Reconstr Surg*, 11: 169-72.

Kim, J., and S. H. Lee. 2012. 'Therapeutic results and safety of postoperative radiotherapy for keloid after repeated Cesarean section in immediate postpartum period', *Radiat Oncol J*, 30: 49-52.

Ledon, J. A., J. Savas, K. Franca, A. Chacon, and K. Nouri. 2013. 'Intralesional treatment for keloids and hypertrophic scars: a review', *Dermatol Surg*, 39: 1745-57.

Lee, S. Y., and J. Park. 2015. 'Postoperative electron beam radiotherapy for keloids: treatment outcome and factors associated with occurrence and recurrence', *Ann Dermatol*, 27: 53-8.

Lee, Y. S., Y. C. Liang, P. Wu, D. A. Kulber, K. Tanabe, C. M. Chuong, R. Widelitz, and T. L. Tuan. 2019. 'STAT3 signalling pathway is implicated in keloid pathogenesis by preliminary transcriptome and open chromatin analyses', *Exp Dermatol*, 28: 480-84.

Mari, W., S. G. Alsabri, N. Tabal, S. Younes, A. Sherif, and R. Simman. 2015. 'Novel Insights on Understanding of Keloid Scar: Article Review', *J Am Coll Clin Wound Spec*, 7: 1-7.

Marneros, A. G., J. E. Norris, B. R. Olsen, and E. Reichenberger. 2001. 'Clinical genetics of familial keloids', *Arch Dermatol*, 137: 1429-34.

Murray, J. C., S. V. Pollack, and S. R. Pinnell. 1981. 'Keloids: a review', *J Am Acad Dermatol*, 4: 461-70.

Nakaoka, H., S. Miyauchi, and Y. Miki. 1995. 'Proliferating activity of dermal fibroblasts in keloids and hypertrophic scars', *Acta Derm Venereol*, 75: 102-4.

Nakashima, M., S. Chung, A. Takahashi, N. Kamatani, T. Kawaguchi, T. Tsunoda, N. Hosono, M. Kubo, Y. Nakamura, and H. Zembutsu. 2010. 'A genome-wide association study identifies four susceptibility loci for keloid in the Japanese population', *Nat Genet*, 42: 768-71.

Niessen, F. B., P. H. Spauwen, J. Schalkwijk, and M. Kon. 1999. 'On the nature of hypertrophic scars and keloids: a review', *Plast Reconstr Surg*, 104: 1435-58.

Ogawa, R. 2010. 'The most current algorithms for the treatment and prevention of hypertrophic scars and keloids', *Plast Reconstr Surg*, 125: 557-68.

Ogawa, R., C. Huang, S. Akaishi, T. Dohi, A. Sugimoto, S. Kuribayashi, T. Miyashita, and H. Hyakusoku. 2013. 'Analysis of surgical treatments for earlobe keloids: analysis of 174 lesions in 145 patients', *Plast Reconstr Surg*, 132: 818e-25e.

Ogawa, R., A. Watanabe, B. Than Naing, M. Sasaki, A. Fujita, S. Akaishi, H. Hyakusoku, and T. Shimada. 2014. 'Associations between keloid severity and single-nucleotide polymorphisms: importance of rs8032158 as a biomarker of keloid severity', *J Invest Dermatol*, 134: 2041-43.

Phan, T. T., I. J. Lim, B. H. Bay, R. Qi, M. T. Longaker, S. T. Lee, and H. Huynh. 2003. 'Role of IGF system of mitogens in the induction of fibroblast proliferation by keloid-derived keratinocytes in vitro', *Am J Physiol Cell Physiol*, 284: C860-9.

Ragoowansi, R., P. G. Cornes, A. L. Moss, and J. P. Glees. 2003. 'Treatment of keloids by surgical excision and immediate postoperative single-fraction radiotherapy', *Plast Reconstr Surg*, 111: 1853-9.

Reish, R. G., and E. Eriksson. 2008. 'Scar treatments: preclinical and clinical studies', *J Am Coll Surg*, 206: 719-30.

Sclafani, A. P., L. Gordon, M. Chadha, and T. Romo, 3rd. 1996. 'Prevention of earlobe keloid recurrence with postoperative corticosteroid injections versus radiation therapy: a randomized, prospective study and review of the literature', *Dermatol Surg*, 22: 569-74.

Shaffer, J. J., S. C. Taylor, and F. Cook-Bolden. 2002. 'Keloidal scars: a review with a critical look at therapeutic options', *J Am Acad Dermatol*, 46: S63-97.

Shin, J. Y., J. W. Lee, S. G. Roh, N. H. Lee, and K. M. Yang. 2016. 'A Comparison of the Effectiveness of Triamcinolone and Radiation Therapy for Ear Keloids after Surgical Excision: A Systematic Review and Meta-Analysis', *Plast Reconstr Surg*, 137: 1718-25.

Shons, A. R., and B. H. Press. 1983. 'The treatment of earlobe keloids by surgical excision and postoperative triamcinolone injection', *Ann Plast Surg*, 10: 480-2.

Smith, J. C., B. E. Boone, S. R. Opalenik, S. M. Williams, and S. B. Russell. 2008. 'Gene profiling of keloid fibroblasts shows altered expression in multiple fibrosis-associated pathways', *J Invest Dermatol*, 128: 1298-310.

Son, Y. and A. Gunjan. 2022a. 'An integrated genomics approach identifies multiple targets for combination drug therapy for keloids.', *About to be submitted.*

Son, Y., E. O. N. Phillips, K. M. Price, L. Z. Rosenberg, B. Stefanovic, C. M. Wolfe, T. S. Shaath, A. Om, G. F. Cohen, and A. Gunjan. 2020. 'Treatment of keloids with a single dose of low-energy superficial X-ray radiation to prevent recurrence after surgical excision: An in vitro and in vivo study', *J Am Acad Dermatol*, 83: 1304-14.

Son, Y., Zamani, N., and A. Gunjan. 2022b. 'Molecular biomarkers for predicting patient response to steroid therapy. ', *About to be submitted.*

Teofoli, P., S. Barduagni, M. Ribuffo, A. Campanella, O. De Pita, and P. Puddu. 1999. 'Expression of Bcl-2, p53, c-jun and c-fos protooncogenes in keloids and hypertrophic scars', *J Dermatol Sci*, 22: 31-7.

Tirgan, MH. 2017. 'Intralesional triamcinolone acetonide in the treatment of keloid lesions-can the treatment be harmful to some patients? results of an online survey', *Int J Keloid Res*, 1: 21-28.

Tirgan, Michael. 2013. "Worsening of keloids after intralesional injections." In *Journal of the American Academy of Dermatology*, AB68-AB68. MOSBY-ELSEVIER 360 PARK AVENUE SOUTH, NEW YORK, NY 10010-1710 USA.

Tosa, M., M. Ghazizadeh, H. Shimizu, T. Hirai, H. Hyakusoku, and O. Kawanami. 2005. 'Global gene expression analysis of keloid fibroblasts in response to electron beam irradiation reveals the involvement of interleukin-6 pathway', *J Invest Dermatol*, 124: 704-13.

Ud-Din, S., A. Bowring, B. Derbyshire, J. Morris, and A. Bayat. 2013. 'Identification of steroid sensitive responders versus non-responders in the treatment of keloid disease', *Arch Dermatol Res*, 305: 423-32.

Wilson, A. M. 2013. 'Eradication of keloids: Surgical excision followed by a single injection of intralesional 5-fluorouracil and botulinum toxin', *Can J Plast Surg*, 21: 87-91.

Zhu, F., B. Wu, P. Li, J. Wang, H. Tang, Y. Liu, X. Zuo, H. Cheng, Y. Ding, W. Wang, Y. Zhai, F. Qian, W. Wang, X. Yuan, J. Wang, W. Ha, J. Hou, F. Zhou, Y. Wang, J. Gao, Y. Sheng, L. Sun, J. Liu, S. Yang, and X. Zhang. 2013. 'Association study confirmed susceptibility loci with keloid in the Chinese Han population', *PLoS One*, 8: e62377.