Beyond the semi-synthetic artemisinin: metabolic engineering of plant-derived anti-cancer drugs
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The discovery and supply of plant-derived anti-cancer compounds remain challenging given their low bioavailability and structural complexity. Reconstituting the pathways of these compounds in heterologous hosts is a promising solution; however, requires the complete elucidation of the biosynthetic genes involved and extensive metabolic engineering to optimise enzyme activity and metabolic flux. This review describes the current strategies and recent advancements in the production of these valuable therapeutic compounds, and highlights plant-derived immunomodulators as an emerging class of anti-cancer agents.

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Identification of missing enzymes in plant specialised metabolism
Biosynthetic pathways of plant-derived anti-cancer NPs are often complex — for example, there are over 31 known enzymatic steps from the primary metabolite geranyl diphosphate to the therapeutic compound vinblastine in the source plant Madagascar periwinkle (Catharanthus roseus). Identification of biosynthetic enzymes remains a major bottleneck in pathway reconstitution; however, recent methodological advancements have accelerated their discovery.

Plant-derived anti-cancer drugs: a recurring problem of supply
Plants produce a chemically diverse range of anti-cancer compounds — the major classes of which are summarised in Table 1, and their therapeutic properties have been reviewed elsewhere [1]. Cancer is a leading cause of mortality, resulting in a high demand for novel therapeutics. Recently, plant-derived immunomodulators have emerged as a new class of anti-cancer compounds. Unfortunately, many plant-derived anti-cancer compounds are synthesised and accumulated at low quantities in planta. Additionally, their structural complexity renders them inviable for mass-production by chemical synthesis. Therefore, to meet supply demands, recent efforts have focused on the production of these natural products (NPs) in heterologous hosts (Figure 1). Re-engineering of host organisms to express native plant biosynthetic enzymes allows de novo synthesis of anti-cancer compounds. Here we discuss the current state and limitations of the elucidation and heterologous reconstitution of plant biosynthetic pathways to produce endogenous and novel therapeutic NPs, with an emphasis on emerging immunomodulatory anti-cancer compounds.
Table 1

Overview of major classes of plant-derived anti-cancer compounds

| Compound class                  | Anticancer agents                  | Plant derived from           | Anti-cancer activity                                      | Reference |
|---------------------------------|------------------------------------|------------------------------|-----------------------------------------------------------|-----------|
| Monoterpane indole alkaloids (MIAs) | Vincristine, vinblastine           | *Catharanthus roseus*        | Anti-mitotic - microtubule destabilising agents           | [1]       |
| Taxane derivatives              | Camptothecin                        | *Camptotheca acuminata*      | DNA damage inducers                                        | [2]       |
| Lignan derivatives              | Paclitaxel, docetaxel, cabazitaxel  | *Taxus genus*                | Anti-mitotic – microtubule stabilising agents             | [1]       |
| Benzyliisoquinoline alkaloids   | Etoposide, teniposide               | *Podophyllum hexandrum*      | DNA damage inducers                                        | [3]       |
|                                 | Noscapine                           | *Papaver somniferum*         | Anti-mitotic – microtubule binding agent                  | [1]       |

Figure 1

Overview of approaches discussed in this review that are used to discover biosynthetic enzymes, and reconstitute and optimise natural product pathways in heterologous hosts to produce anti-cancer compounds. Created with Biorender.com.
Once candidate enzymes have been selected, their enzymatic activities are characterised using functional assays in conjunction with metabolomic techniques to detect product formation. While being efficient, such assays can be complicated due to unknown or unpurifiable reaction substrates and therefore requiring the use of either crude whole-plant or tissue extracts. Alternatively, virus-induced gene silencing (VIGS) can be used to characterise gene function in vivo, but requires plant-specific development as demonstrated in *C. roseus* and *Camptotheca acuminata* [14,15].

**Selecting a suitable heterologous host organism**

Biosynthetic gene discovery enables engineering of NP pathways to produce pharmaceutically valuable compounds. A key factor in successful pathway reconstitution is the choice of an appropriate heterologous host based on the host’s characteristics, as summarised in Table 2. Plant NP pathway reconstitution poses a unique challenge due to the often high number of enzymatic steps, complex tissue and subcellular localisation of intermediates, and intricate regulation networks, exemplified in the vinblastine biosynthetic pathway [7]. In addition, the scalability and economic-viability of production must be considered, taking into account the choice of substrate and the ease of downstream product purification.

The bacterium *Escherichia coli* is a common heterologous host for plant NP biosynthesis due to its established genetics toolbox, well-understood native metabolism and fast doubling time. *E. coli* has been used for the production of many NPs including taxadene and reticuline [19,20]. However, there are significant physiological differences between plants and *E. coli*, such as the lack of eukaryotic cell architecture and protein post-translational modification mechanisms. These differences can disrupt the metabolic flux of the reconstituted pathway and reduce product titres, as demonstrated by the transfer of semi-synthetic artemisinin production from *E. coli* into the eukaryotic *Saccharomyces cerevisiae* host [21].

*S. cerevisiae* is a typical eukaryotic host that has been used in the reconstitution of cannabinoids, noscapine and monoterpene indole alkaloids (MIA) pathways [22*,23,24*,25,26], and its use has been extensively reviewed elsewhere [17]. A key advantage is its eukaryotic cell architecture, allowing the expression of membrane-localised proteins commonly found in NP pathways such as cytochrome P450’s [27].

*Nicotiana benthamiana* is the most widely used plant chassis for heterologous NP biosynthesis; however, alternative systems such as the false flax *Camelina sativa*, microalgae, and the moss *Physcomitrella patens* have also been used [28–30]. Plant systems have the advantages of better replicating native pathway compartmentalisation at both the tissue and cellular levels, and have the required cofactors and post-translational mechanisms for proper enzyme expression and activity [18]. Unfortunately, the limited number of developed genetic modification tools and the organism’s longer generation time hamper their use in NP pathway reconstitution.

The use of hosts with edited or minimal synthetic genomes is currently being explored to improve product yields compared to their native host counterparts [31,32]. Gene editing allows the targeting of destabilising elements such as enzymes that derivatise biosynthetic precursors [33]. Minimal synthetic genome organisms have been developed that only retain genetic elements of known function, greatly decreasing the risk of metabolic flux shifting [34]. This enables impact modelling of the introduced heterologous pathway on native host metabolism, allowing for better design of the reconstitution process [35,36]. However, due to the complexity of higher plants, synthesis of minimal genomes is currently only available in microbial organisms.

**Heterologous reconstitution of plant biosynthetic pathways**

Recent advances in molecular techniques have accelerated the heterologous reconstitution of NP pathways. Technologies such as Golden Gate cloning and Gibson assembly enable segmentation of pathways into clonable modules [37,38]. In accordance with these systems, syntaxes containing standardised parts have been developed for their use in different hosts [37,39]. Empirical combinations of standardised parts, especially regulatory elements, can then be tested to optimise pathway flux. Screening of resulting genetic modules can be further accelerated through the use of automated biofoundries based on the design-build-test (DBT) cycle, outlined in Figure 2 and reviewed elsewhere [40].

Novel genome editing technologies have increased the ease of integrating longer pathways into a host’s genome. Combinatorial toolkits such as the Easy-Clone system use homologous recombination and CRISPR-Cas for markerless, single-step genome integration of multiple biosynthetic modules into pre-defined sites [41]. This approach lowers the risk of recombination within the target constructs, and thus the loss of their parts [42]. The hierarchical organisation of constructs and availability of online regulatory sequence libraries allows for easy selection and swapping of parts in *S. cerevisiae* [43,44].

Transient expression remains the most widely used reconstitution platform in plant hosts, successfully producing medically-active terpenes [45**]. *Agrobacterium*-mediated co-infiltration of plants allows rapid expression of biosynthetic enzymes. However, the system’s lack of protein stoichiometry control and the limited number of genes that can be expressed make it less suitable for reconstituting longer pathways [18]. A possible solution to
these challenges may lie in the stacking of target biosynthetic genes into a single vector [39].

Because of the complexity of their genomes, gene-editing technologies in plant systems are still being developed. The relative simplicity of plastomes compared to their nuclear counterparts renders them more amenable to gene editing and therefore these have been targeted for NP pathway reconstitution [46]. However, recent developments in CRISPR-Cas technologies have enabled whole plant genome editing to eliminate endogenous NP derivatives [47]. Although still in their infancy, these gene-editing techniques pose exciting prospects for the future of de novo production of plant-derived NPs.

**Metabolic flux and process optimisation**

Heterologous reconstitution of long pathways often causes metabolic cellular stress, reducing host growth and thereby production efficiency. Low or absent activity in bottleneck enzymes can lead to the build-up of pathway intermediates, thereby affecting cell fitness. Protein engineering and the introduction of suitable exogenous enzymes are commonly used strategies to improve enzyme activity, alleviating bottlenecks and thereby increasing pathway flux [24]. The identification and modulation of protein–protein interactions are also important factors, as exemplified by the engineering of cytochrome P450 enzymes and their reductase partner to improve oxygenated taxane production [48]. An alternative strategy is to reduce the spatial dispersion of pathway intermediates by fusing downstream enzymes, as achieved in taxol and geraniol biosyntheses [19,49*].

The availability of metabolic precursors or enzymatic cofactors is also an important component in metabolic flux optimisation. In the recent cannabinoid biosynthesis reconstitution, engineered upregulation of the mevalonate pathway in primary metabolism increased the metabolic flux towards the target NP pathway [21]. Precursor availability can also be increased by knocking-down non-essential competing enzymes, or by degron-tagging essential competing enzymes [24,50]. The presence of growth media additives such as glycerol may also improve cofactor availability, demonstrated in the reconstitution of the noscapine pathway in *S. cerevisiae* [24*].

Heterologous NP biosynthesis can also be hampered by improper host post-translational modification mechanisms, causing protein misfolding or mistargeting and thereby affecting enzyme activity. Modification or addition of localisation sequences is a commonly used strategy to improve enzyme targeting within the host at both the cellular and subcellular levels. This prevents the loss of pathway intermediates by diffusion and reduces metabolic flux into undesired pathways [21,24*]. This technique was exemplified in the recent targeted production of the MIA precursor geraniol in *S. cerevisiae* mitochondria, whereby exploiting the host’s lack of intermediate-specific catabolic enzymes in the organelle enabled increased intermediate accumulation and thereby higher end-product titres [49*].

### Table 2

| Organism Domain | Heterologous host | Advantages | Disadvantages | Ref. |
|-----------------|-------------------|------------|--------------|------|
| Prokaryotic     | Bacteria for example, *Escherichia coli* | - Fast generation times  
- Well established genetic toolboxes  
- Efficient gene-editing tools  
- Ease of culturing on cheap carbon sources | - Lack of eukaryotic cell architecture for expression of plant enzymes  
- Lack of post-translational modifications needed for expression of plant enzymes  
- Lack of certain biosynthetic precursors and co-factors | [16] |
| Eukaryotic      | Yeasts for example, *Saccharomyces cerevisiae* | - Fast generation times  
- Well-established genetic toolboxes  
- Efficient gene-editing tools  
- Ease of culturing on various cheap carbon sources | - Lack of tissue compartmentalisation (single cell organism)  
- Different post-translational mechanisms compared to plants | [17] |
| Plants for example, *Nicotiana benthamiana* | - Eukaryotic cell architecture  
- Pathway compartmentalisation possible at both tissue and cellular level  
- Presence of relevant post-translational modifications and enzyme co-factors  
- Possibility of rapid transient expression of pathways  
- No need for exogenous carbon source | - Longer generation times  
- Lack of well-established genetic toolboxes  
- Efficient genome-editing tools still at early stages of development  
- Lack of detailed knowledge of metabolism  
- Costly growth facilities and upscaling of production | [18] |
Intermediate localisation and accumulation can also be manipulated by transporter protein engineering; however, few have been identified in plant NP pathways. In heterologous hosts, this technique has been used to introduce the uptake of alternative carbon sources [51]. Additionally, transporter engineering can increase flux into a biosynthetic pathway by reducing the production of unwanted by-products as exemplified by the fusion of a xylose transporter to a xylose isomerase [52].

**New-to-nature plant-derived anti-cancer compounds**

Advances in heterologous pathway reconstitution have recently turned to the production of new-to-nature plant-derived compounds to expand metabolic diversity. A common strategy to produce novel NPs is by reengineering or exploiting an enzyme’s natural substrate promiscuity to allow the feeding of non-natural precursors. Feeding of halogenated substrates has produced novel compounds with new or improved pharmacological properties such as in the opiate, MIA and phenylpropanoid pathways [24,53,54]. This technique has also been used in the feeding of fatty acids to produce unnatural cannabinoid analogues [22]. Knowledge of the biosynthetic pathway also enables a combinatorial approach to produce novel NPs by swapping, omitting or introducing new enzymatic steps in a non-canonical fashion. This was exemplified by the use of non-native cytochrome P450 enzymes to oxidise β-amyrin in *N. benthamiana*, resulting in novel triterpenes [45].
Plant-derived immunomodulatory agents: an emerging class of anti-cancer compounds

Plant-derived immunomodulatory agents are an emerging class of anti-cancer compounds that are currently largely unexploited due to their low bioavailability. Unlike cytotoxic anti-cancer compounds (summarised in Table 1), immunomodulators target specific constituents of the cancer development process through various mechanisms, outlined in Table 3. Detailed mechanisms of major plant-derived immunomodulators have been reviewed elsewhere [55]. In clinical trials, these compounds show high cell type interaction specificity, potentially enabling more predictable and targeted treatments [56]. Immunosuppressants such as artemisinin and its derivatives including artemisone, artesunate, and dihydroartemisinin are being investigated as tumour-inhibiting therapeutics due to their modulation of various signalling cascades involved in the spread of cancer [57]. Matrine is another example of a plant-derived immunosuppressant that acts by inducing the expression of anti-tumour immune defence activating ligands [60,61]. Plant-derived immunostimulants such as curcumin stimulate immune cell production involved in tumour defence, increasing immune system identification of tumour cells and the subsequent activation of apoptotic pathways [59]. The alkaloid tryptanthrin is also an effective stimulant of T cell recruitment, triggering the immune inflammatory response against cancer cells [61].

As the biosynthesis of more NPs are elucidated, new potentially therapeutic chemical scaffolds are becoming available. The recent elucidation and heterologous reconstitution of the immunosuppressant alkaloid berberine enables the production and development of novel derivatives with improved pharmacokinetic properties [58,17]. Although this class of anti-cancer therapeutics remains understudied, use of NP pathway discovery and reconstitution techniques will enable advances towards the production of novel immunomodulatory compounds. Reaching a high-level production of these compounds will require complementary pathway engineering. For terpenoid compounds, a fine tuning of the metabolic flux can be obtained by limiting competing metabolic branches through the downregulation of the expression of the yeast gene ERG9 for instance. The resulting rerouting of the flux towards isopentenyl diphosphate (IPP) thus favours arte-misinic acid production by decreasing ergosterol synthesis [62]. Engineering of enzyme specificity and efficiency for acceptance of orthogonal substrates that do not compete directly with endogenous pathways of the host organism can be also explored as recently demonstrated for monoterpenoid biosynthesis in yeast [63].

Concluding remarks

Significant developments over the last few years have enabled the heterologous reconstitution of increasingly complex plant biosynthetic pathways to produce anti-cancer compounds. Improvements in gene discovery techniques in conjunction with sophisticated synthetic engineering tools such as genome editing have accelerated the process of pathway elucidation and heterologous reconstitution. Increased screening of plant-derived NPs has revealed new classes of anti-cancer therapeutics such as immunomodulatory compounds. Despite recent biotechnological improvements, significant limitations remain, preventing the mass-production of these plant-derived NPs in heterologous systems. Securing and increasing the supply of these compounds are vital to meet both current demands, and will also expand the panel of highly toxic compounds used to generate antibody drug conjugates for immunotherapies.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest

•• of outstanding interest

1. Agarwal G, Carcach PJB, Addo EM, Kinghorn AD: Current status and contemporary approaches to the discovery of
antitumor agents from higher plants. Biotechnol Adv 2019
http://dx.doi.org/10.1016/j.biotechadv.2019.01.004.

2. Martino E, Della Volpe S, Terrible E, Benetti E, Sakaj M,
Centamore A, Sala A, Collina S: The long story of camptothecin:
from traditional medicine to drugs. Bioorg Med Chem Lett 2017,
27:701-707.

3. Ardalani H, Ayan A, Ghayour-Mobaran M: Podophyllotoxin: a
novel potential natural antitumor agent. Avicenna J Phytomed 2017,
7:285-294.

4. Caputi L, Franke J, Farow SC, Chung K, Payne RME, Nguyen TD,
Dang TT, Carqueijeiro IST, Koudounas K, de Bernoville TD et al.: Missing
enzymes in the biosynthesis of the antitumor drug vinblastine in
Madagascar periwinkle. Science 2018, 360:1235-1238

The authors identified the missing enzymes of tabersonine/catharanthine
biosynthetic pathway thus ending more than 60 years of research on the Mada-
gascar periwinkle and opening new perspectives towards the production of
antitumor monoterpene indole alkaloids in heterologous organisms.

5. Lau W, Sattely ES: Six enzymes from mayapple that complete
the biosynthetic pathway to the etoposide aglycone. Science 2015,
349:S1224-1228.

6. Payne RME, Xu DY, Fouere E, Carqueijeiro MIST, Oudin A, de
Bernoville TD, Novak V, Burow M, Olsen CE, Jones DM et al.: An NPF
transporter exports a central monoterpene indole alkaloid intermediate
from the vacuole. Nat Plants 2017, 3.

7. Yamamoto K, Takahashi K, Caputi L, Mizuno H, Rodriguez-
Lopez CE, Iwasaki T, Ishizaki F, Fukui H, Ohnishi M, Yamazaki M et al.: The complexity of intercellular localisation of alkaloids
revealed by single-cell metabolomics. New Phytol 2019,
224:848-859.

8. Kautsar SA, Duran HGS, Blik N, Osbourn A, Medema MH:
PlantiSMASH: automated identification, annotation and expression
analysis of plant biosynthetic gene clusters. Nucleic Acids Res 2017,
45:W55-W63.

9. Guo L, Winzer T, Yang XF, Li Y, Ngin ZM, He ZS, Teodor R, Lu Y,
Bowser TA, Graham IA et al.: The opium poppy genome and
morphinan production. Science 2018, 362:343.

10. Kellner F, Kim J, Clavijo BJ, Hamilton JP, Childs KL, Vaillancourt B,
Cepela J, Habermann M, Steuernagel B, Cissoldi L et al.: Genome-guided
investigation of plant natural product biosynthesis. Plant J 2015,
82:680-692.

11. Zhao D, Hamilton JP, Pham GM, Crisovan E, Wiertgen-Rininger K,
Vaillancourt B, Dellapenna D, Robin Buell C: De novo genome
assembly of Delphium acuminatum, a natural source of the
anti-cancer compound camptothecin. GigaScience 2017, 6.

12. Farrow SC, Kamileen MO, Caputi L, Bussey KM, Mundy JEA,
McAtee RC, Stephenson CRJ, O’Connor SE: Biosynthesis of an
anti-addiction agent from the iboga plant. J Am Chem Soc 2019,
141:12979-12983.

13. Franke J, Kim J, Hamilton JP, Zhao D, Pham GM, Wiertgen-
Rininger K, Crisovan E, Newton L, Vaillancourt B, Tatsis E et al.: Gene
discovery in Gelsemium highlights conserved gene clusters
in monoterpene indole alkaloid biosynthesis. ChemBioChem 2019,
20:83-87.

14. Sung YC, Lin CP, Chen JC: Optimization of virus-induced gene
silencing in Catharanthus roseus. Plant Pathol 2014, 63:1159-1167.

15. Jin Z, Cong Y, Zhu S, Zhang D, Yao X, Wan R, Wang Y, Yu F: Two
classes of cytochrome P450 reductase genes and their
divergent functions in Campotheca acuminata Decne. Int J
Biol Macromol 2019, 138:1098-1108.

16. Baaschen MN, Al-Heij AM, Bora RS, Ahmed MM, Ramadan HA,
Saini KS, Baaschen NA, Redwan EM: Production of biopharmaceuticals in E. coli: current scenario and future
perspectives. J Microbiol Biotechnol 2015, 25:953-962.

17. Cravens A, Payne J, Smolke CD: Synthetic biology strategies
for microbial biosynthesis of plant natural products. Nat Commun
2019, 10:2142.

18. Reed J, Osbourn A: Engineering terpenoid production through
transient expression in Nicotiana benthamiana. Plant Cell Rep 2018,
37:1431-1441.

19. Aijkumar PK, Xiao WH, Tyo KEJ, Wang Y, Simeen F, Leonard E,
Mocha O, Phom TH, Pfeifer B, Stephanopoulos G: Isoprenoid
pathway optimization for taxol precursor overproduction in
Escherichia coli. Science 2010, 330:70-74.

20. Matsumura E, Nakagawa A, Tomobechi Y, Iiokuro S, Sakaki T,
Katayama Y, Yamamoto K, Kumagai H, Sato F, Minami H: Microbial
transformation of novel sulphated alkaloids for drug
discovery. Sci Rep 2018, 8.

21. Paddon CJ, Keasling JD: Semi-synthetic artemisinin: a model
for the use of synthetic biology in pharmaceutical development.
Nat Rev Microbiol 2014, 12:355-367.

22. Luo X, Reiter MA, d’Espaux L, Wong J, Denby CM, Lechner A,
Zhang Y, Grzybowski AT, Hart S, Lin W et al.: Complete
biosynthesis of cannabinoids and their unnatural analogues in yeast.
Nature 2019, 567:123-126

The authors not only achieved total biosynthesis of cannabinoids in yeast
but also production of non-natural analogue compounds by feeding the strain
directly with precursor.

23. Carqueijeiro I, Duque de Bernoville T, Lanoue A, Dang TT,
Teijaro CN, Paetz C, Billet K, Mosquera A, Oudin A, Besseau S et al.: A BAHD acyltransferase catalyzing 19-O-acetylation of
tabersonine derivatives in roots of Catharanthus roseus enables combinatorial synthesis of monoterpene indole
alkaloids. Plant J 2018, 94:469-484.

24. Li Y, Li S, Thodey K, Trenchard I, Cravens A, Smolke CD: Complete
biosynthesis of noscapine and halogenated alkaloids in yeast.
Proc Natl Acad Sci U S A 2018, 115:E3922-E3931

The authors successfully reconstitute the noscapine pathway in S. cer-
evise and achieve impressive titres of the final product through the
optimisation of metabolic flux, precursor overproduction and adjustment of
fermentation conditions.

25. Brown S, Clastre M, Courdavault V, O’Connor SE: De novo
production of the plant-derived alkaloid strictosidine in yeast.
Proc Natl Acad Sci U S A 2015, 112:3205.

26. Qu Y, Easson MLA, Froese J, Simionescu R, Hudlicky T, De Luca V:
Completion of the seven-step pathway from tabersonine to the antitumor
drug precursor vindoline and its assembly in yeast.
Proc Natl Acad Sci U S A 2015, 112:6224-6229.

27. Nelson D, Werck-Reichhart D: A P450-centric view of plant
evolution. Plant J 2011, 66:194-211.

28. Augustin MM, Shukla AK, Starks CM, O’Neill-Johnson M, Han L,
Holland CK, Kutchan TM: Biosynthesis of Veratrumin californicum
speciality chemicals in Camelina sativa seed.
Plant Biotechnol Rep 2017, 11:29-41.

29. Poliner E, Farré EM, Benning C: Advanced genetic tools enable
synthetic biology in the oleaginous microalga
Nannochloropsis sp. Plant Cell Rep 2018, 37:1383-1399.

30. Reski R, Bae H, Simonsen HT: Physcomitrella patens, a versatile
synthetic biology chassis. Plant Cell Rep 2018, 37:1409-1417.

31. Richardson SM, Mitchell LA, Stracquadanio G, Yang K, Dymond JS, DiCarlo JE, Lee D, Huang CL, Chandrasegaran S,
Cai Y et al.: Design of a synthetic yeast genome. Science 2017,
355:1040-1044.

32. Fredens J, Wang K, de la Torre D, Funke LFH, Robertson WE,
Christova Y, Chia T, Schmied WH, Dunkelmenn DL, Beranek V et al.: Total synthesis of Escherichia coli with a recorded
genome. Nature 2019, 569:514-518.

33. Janss J, Sack M, Augustine SM, Fischer R, Bortesi L: CRISPR/
Cas9-mediated knockout of six glycosyltransferase genes in
Nicotiana benthamiana for the production of recombinant
proteins lacking beta-1,2-xylose and core alpha-1,3-fucose.
Plant Biotechnol J 2019, 17:350-361.

34. Glass JI, Merryman C, Wise KS, Hutchison. CA 3rd, Smith HO:
Minimal cells-real and imagined. Cold Spring Harbor Perspect Biol
2017, 9.

35. Lu H, Villada JC, Lee PKH: Modular metabolic engineering
for biobased chemical production. Trends Biotechnol 2019, 37:152-166.

36. Lloyd CJ, Ebrahim A, Yang L, King ZA, Catouli E, O’Brien EJ, Liu JK,
Palsson BO: COBRAME: a computational framework for
genome-scale models of metabolism and gene expression. PLoS Comput Biol 2018, 14:e1006302.

37. Engler C, Youles M, Gruetnzer R, Ehert TM, Werner S, Jones JD, Patron NJ, Maniljonnet S: A golden gate modular cloning toolbox for plants. ACS Synth Biol 2014, 3:839-843.

38. Gibson DG, Young L, Chuang RY, Venter JC, Hutchison CA, Smith HO: Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat Methods 2009, 6:343-U341.

39. Shih PM, Vuu K, Mansoori N, Ayad L, Louie KB, Bowen BP, Northen TR, Loque D: A robust gene-stacking method utilizing yeast assembly for plant synthetic biology. Nat Commun 2016, 7:13215.

40. Chao R, Mishra S, Si T, Zhao HM: Engineering biological systems using automated biofoundries. Metab Eng 2017, 42:98-108.

41. Jakobiunas T, Rajkumar AS, Zhang J, Arsovskia D, Rodriguez A, Jendresen CB, Skjold ML, Nielsen AT, Borodina I, Jensen MK, et al.: CasEMBLR: Cas9-facilitated multiloci genomic integration of in vivo assembled DNA parts in Saccharomyces cerevisiae. ACS Synth Biol 2015, 4:1226-1234.

42. Stovichek V, Holkenbrink C, Borriona I: CRISPR/Cas system for yeast genome engineering: advances and applications. FEMS Yeast Res 2017, 17 fox030.

43. Dossani ZY, Reider Apel A, Szmidt-Middleton H, Hiltson NJ, Deutsch S, Keasing JD, Mukhopadhyay A: A combinatorial approach to synthetic transcription factor-promoter combinations for yeast strain engineering. Yeast 2018, 35:273-280.

44. Kotopka BJ, Smolke CD: Model-driven generation of artificial yeast promoters. bioRxiv 2019:748616.

45. Reed J, Stephenson MJ, Mittinnen K, Brouwer B, Leveau A, Bret P, Goss RJM, Goossens A, O’Connell MA, Osbourn A: A translational synthetic biology platform for rapid access to gram-scale quantities of novel drug-like molecules. Metab Eng 2017, 42:185-193.

The study demonstrates a rapid combinatorial method for production of novel terpenes with potential medicinal activities, illustrating how N. benthamiana can be used not only for quick testing of enzyme combinations, but also for industrial-scale production of bio-pharmaceuticals.

46. Boehm CR, Bock R: Recent advances and current challenges in synthetic biology of the plant genetic system and metabolism. Plant Physiol 2019, 179:794.

47. Schachtisiek J, Stehle F: Nicotine-free, nonnursigenic tobacco (Nicotiana tabacum L) edited by CRISPR-Cas9. Plant Biotechnol J 2019, 17:2228-2230.

48. Biggs BW, Lim CG, Saglani K, Shankar S, Stephanopoulos G, De Muy M, Ajikumar PK: Overcoming heterologous protein interdependency to optimize P450-mediated Taxol precursor synthesis in Escherichia coli. Proc Natl Acad Sci U S A 2016, 113:3209.

49. Yee DA, DeNicola AB, Billingsly JM, Creso JG, Subrahmanyan V, Tang Y: Engineered mitochondrial production of monoterpene in Saccharomyces cerevisiae. Metab Eng 2019, 55:76-84.

The study demonstrates targeting of geraniol biosynthesis to S. cerevisiae mitochondria to reduce derivatisation of pathway intermediates into unwanted side-products and thereby increase end-product titre of nepetalactol.

50. Peng BY, Nielsen DK, Kampranis SC, Vickers CE: Engineered protein degradation of farnesyl pyrophosphate synthase is an effective regulatory mechanism to increase monoterpene production in Saccharomyces cerevisiae. Metab Eng 2018, 47:83-93.

The study highlights an interesting approach to metabolic flux re-directing by degradation of enzymes essential to the survival of the heterologous host, but disadvantageous to the yields of the target pathway.

51. Yaguchi A, Spagnuolo M, Binner M: Engineering yeast for utilization of alternative feedstocks. Curr Opin Biotechnol 2018, 53:122-129.

52. Thomkik T, Witting I, Choe JY, Boles E, Oreb M: An artificial transport metabolon facilitates improved substrate utilization in yeast. Nat Chem Biol 2017, 13:1158.

53. Runguphan W, Qu X, O’Connor SE: Integrating carbon-halogen bond formation into medicinal plant metabolism. Nature 2010, 468:461-464.

54. Wang S, Zhang S, Xiao A, Rasmussen M, Skidmore C, Zhan J: Metabolic engineering of Escherichia coli for the biosynthesis of various phenylpropanoid derivatives. Metab Eng 2015, 29:153-159.

55. Jantan I, Ahmad W, Bukhari S A: Plant-derived immunomodulators: an insight on their preclinical evaluation and clinical trials. Front Plant Sci 2015, 6:655.

56. Mohamed SIA, Jantan I, Haque MA: Naturally occurring immunomodulators with antitumor activity: an insight on their mechanisms of action. Int Immunopharmacol 2017, 50:291-304.

57. Yao W, Wang F, Wang H: Immunomodulation of artemisinin and its derivatives. Sci Bull 2016, 61:1399-1406.

58. Tillhon M, Guaman Ortiz LM, Lombardi P, Scovassi AI: Berberine: new perspectives for old remedies. Biochem Pharmacol 2012, 84:1260-1267.

59. Bose S, Panda AK, Mukherjee S, Sa G: Curcumin and tumor immune-editing: resurrecting the immune system. Cell Div 2015, 10:6.

60. Lu X, Zhu Z, Jiang L, Sun X, Jia Z, Qian S, Li J, Ma L: Matrine increases NKG2D ligand ULBP2 in KS62 cells via inhibiting JAK/STAT3 pathway: a potential mechanism underlying the immunotherapy of matrine in leukemia. Am J Transl Res 2015:1838-1849.

61. Yang S, Li X, Hu F, Li Y, Yang Y, Yan J, Kuang C, Yang Q: Discovery of tryptanthrin derivatives as potent inhibitors of indoleamine 2,3-dioxygenase with therapeutic activity in Lewis Lung Cancer (LLC) tumor-bearing mice. J Med Chem 2013, 56:8321-8331.

62. Paddock CJ, Westfall PJ, Pitera DJ, Benjamin K, Fisher K, McPhee D, Leavell MD, Tai A, Main A, Eng D, Polichuk DR et al.: High-level semi-synthetic production of the potent antimalarial artemisinin. Nature 2013, 496:528-532.

63. Ignea C, Raadam MH, Motawia MS, Makris AM, Vickers CE, Kampranis SC: Orthogonal monoterpene biosynthesis in yeast constructed on an isomeric substrate. Nat Commun 2019, 10:3799.