Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Pharmacology

Targeting microRNAs for immunomodulation

Lukas T Jeker and Romina Marone

microRNAs (miRNA) are small regulatory RNAs exerting pleiotropic functions in virtually any immune cell-type. Dozens of miRNAs with a known function in the immune system constitute interesting drug targets for immunomodulation. Chemical modifications of nucleic acid-based miRNA mimics and inhibitors largely solved instability issues but delivery to immune cells remains a major challenge. However, recent success targeting the acidic tumor microenvironment is very promising for inflammatory diseases. Moreover, small molecules are being explored as an interesting alternative. Although RNA is often considered 'undruggable' by small molecules recent progress modulating miRNA function through small molecules is encouraging. Computational approaches even allow predictions about specific small molecule/RNA interactions. Finally, recent clinical success demonstrates that drugs targeting RNAs work in humans.

Address
Department of Biomedicine, Basel University Hospital and University of Basel, Hebelstrasse 20, CH-4031 Basel, Switzerland

Corresponding author: Jeker, Lukas T (lukas.jeker@unibas.ch)

Current Opinion in Pharmacology 2015, **23**:25–31

This review comes from a themed issue on **Immunomodulation**

Edited by **Stephen M Anderton** and **Simon Fillatreau**

For a complete overview see the Issue and the Editorial

Available online 25th May 2015

http://dx.doi.org/10.1016/j.coph.2015.05.004

Introduction

Genes encoding proteins have for a long time dominated biomedical research and proteins were the main targets for therapeutic interventions [1]. However, only a small fraction of the mammalian genome actually encodes proteins. Large parts of the genome that does not encode proteins are transcribed and are collectively called 'non-coding' RNAs [2]. In contrast to messenger RNAs (mRNAs), which serve as intermediates to transfer information from DNA to protein, the RNA transcripts themselves are the functional unit of non-coding genes. Various classes of short and long non-coding RNAs control gene expression and the majority of causal genetic variants driving autoimmune diseases are found in non-coding regions illustrating the importance of non-coding DNA [3].

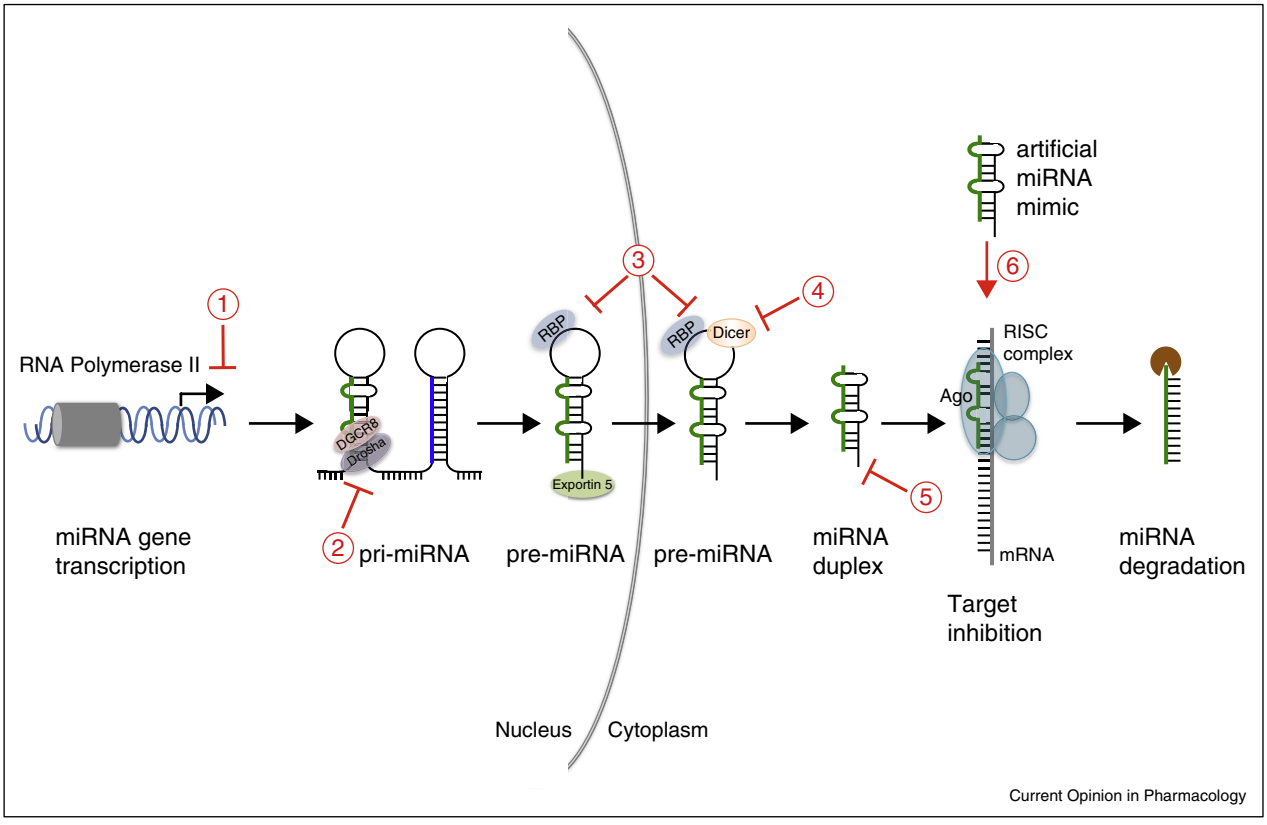
Here we focus on microRNAs (miRNAs), which belong to the best-characterized small non-coding RNAs. We discuss progress published in the past few years in our understanding of miRNA function in the immune system with a particular focus on lymphocytes. It is beyond the scope of this review to discuss each miRNA or immune cell in detail. Instead, we highlight a few examples to illustrate principles that are relevant from a therapeutic perspective. We discuss potential therapeutic options and various approaches to pharmacologically modulate miRNA function to achieve immunomodulation. In principle, the fact that therapeutic interventions have historically focused on proteins means that a lot of other targets have been ignored. Thus, a treasure trove of a wide variety of novel targets opens up. However, we also discuss the challenges that need to be addressed when targeting non-coding genes or their products in the immune system.

microRNAs: important regulators of the immune system

miRNAs are short posttranscriptional gene repressors, which mediate their function through partial Watson-Crick base-pairing with its target RNAs. Canonical miRNAs are transcribed as long primary miRNAs (pri-miRNAs) (Figure 1). The nuclear multiprotein microprocessor complex (Dgcr8 and Drosha) then recognizes and cleaves a hairpin in the pri-miRNA. The released precursor miRNA (pre-miRNA) gets exported to the cytoplasm where further processing occurs through Dicer. Several additional RNA binding proteins (RBPs) such as LIN28 can influence miRNA biogenesis [2,4•]. Ultimately the mature, short miRNA duplex is loaded onto Argonaute (Ago) to form a multiprotein effector complex termed RNA-induced silencing complex (RISC) [4•]. Interaction of the miRNA with its target RNA occurs in RISC and leads to translational inhibition and later mRNA degradation [5•]. Nucleotides 2–7 termed the 'seed' region of a miRNA mostly determine specificity for RNA targets. miRNAs with the same seed region are grouped into families which target the same RNAs. Finally, miRNA stability and turnover are actively controlled by various mechanisms [6,7].

Several hundred miRNA genes exist as individual miRNAs or clustered in the mammalian genome. Genetic ablation of key genes in the miRNA biogenesis pathway (e.g. *Drosha*, *Dgcr8*, *Dicer*, and *Ago*) results in cells devoid of miRNAs. Cell-type specific ablation studies have demonstrated a critical role for miRNAs in all examined immune cells [8–10]. Thus, miRNAs as a class of regulatory short RNAs are critical for normal function of

Figure 1



Pharmacologic intervention strategies to modulate miRNA biogenesis and function. miRNAs are transcribed by polymerase II into primary miRNAs (pri-miRNA) which are processed into precursor miRNAs (pre-miRNA) by Drosha/Dgcr8. After export to the cytoplasm where further processing occurs through Dicer the mature miRNA is loaded onto Argonaute (Ago) in a multiprotein complex termed RNA induced silencing complex (RISC). The multi-step generation of canonical miRNAs allows modulation of miRNA expression using different approaches to interfere with specific steps of miRNA biogenesis. (1) Small molecule inhibitor of transcription [44]. (2) Small molecule pri-miRNA processing inhibitor [48••]. (3) pre-miRNA processing inhibitor: small molecule or looptomir [39••]. (4) Small molecule targeting the processing of pre-miRNA-21 by Dicer [47]. (5) Inhibitors of mature miRNA: Antisense oligonucleotides; miRNA 'sponges'; antagomir; seed targeting LNA-modified antimiRs [28•]. (6) Delivery of artificial miRNA mimic.

immune cell types regulating cell survival, proliferation, differentiation and maintenance of cellular identity.

In contrast, functional characterization of individual miRNAs or miRNA clusters has proven very challenging [5•]. Nevertheless, there is now a large body of literature demonstrating the importance of individual miRNAs regulating different cells of the immune system and associations with human immunologic diseases [8,9,11]. Some miRNAs are transcribed individually whereas others are generated from polycistronic transcripts that encode multiple miRNAs. The miR-17-92 cluster can serve as a paradigm to illustrate concepts that are broadly applicable to other miRNAs as well. The pri-miR-17-92 transcript encodes for 6 different miRNAs with four different seed families. Posttranscriptional processing of the miR-17-92 cluster itself leads to differential expression of individual miRNAs within that cluster and intracluster antagonism is functionally relevant since a shifted balance of antagonistic miRNAs can contribute to pathogenesis [12,13]. Thus, combinatorial effects lead to an enormously diverse context-dependent gene regulatory capacity [2]. Therefore, therapeutic modification of the expression of the entire cluster may result in different effects than modulating the abundance of individual miRNA members or modulation of processing of the cluster.

Promises of therapeutically targeting miRNAs

miRNAs are attractive therapeutic targets because they fulfill several criteria defining a good drug target [1]. Although repression of individual target genes is generally mild, coevolution of miRNAs and their targets led to regulation of entire pathways by individual miRNAs. Hence, modulating miRNA expression promises to regulate entire pathways [14]. Importantly, individual miRNAs can be drivers of disease. As an example, overexpression of miR-17-92 is sufficient to drive lymphomagenesis and a lupus-like lymphoproliferative disease whereas the cluster is required for B and T cell differentiation of various lymphocyte subsets [9,15,16,17•,18•,19•]. Furthermore,

dysregulation of miR-17-92 or individual cluster members is associated with multiple human immunologic diseases [9,14]. To qualify as an ideal drug target a miRNA should be pathogenic and exclusively expressed in a given tissue. For instance miR-122 is highly and specifically found in the liver [2]. Therefore systemic miR-122 blockade will only have on-target effects in hepatocytes. In contrast, no miRNA is known to be exclusively expressed in the immune system although several miRNAs are enriched. However, despite the absence of specific expression pharmacologic blockade of selected miRNAs should be well tolerated by most cells since miRNA-deficient animals frequently do not display overt phenotypes [5•,20]. An important concept proposes that many miRNAs are required for biologic robustness during stress responses [21]. Thus, absence of a given miRNA will be tolerated under homeostatic conditions but environmental perturbations will in some cases result in adaptations that depend on the miRNA. Therefore, during perturbations the lack of a miRNA's fine-tuning regulatory effect can result in observable phenotypic consequences and thus reveal miRNA function in miRNA-deficient organisms [21,22]. Thus, identification of specific biologic processes that depend on a particular miRNA promise to result in preferential targeting of that cell type or process while sparing other cells when the miRNA is inhibited. Tumors can become dependent on the disease-driving miRNA, a process termed oncomiR addiction. Inhibition of the pathogenic miRNA leads to rapid tumor shrinkage [23]. As another example, miR-17-92 is dispensable for development of various T cell subsets under homeostatic conditions and mice harboring miR-17-92-deficient regulatory T cells (Treg) age normally without signs of Treg dysfunction [17•,19•,24,25]. In contrast, induction of an acute immune response results in much more severe disease in mice with miR-17-92-deficient Treg than in mice with miR-17-92-sufficient Treg. Mechanistically, antigen-specific effector Treg were much more affected than the remaining Treg [19•]. Similarly, miR-17-92-deficient $CD4^+$ T cells develop normally but miR-17-92 is required for follicular helper T cell (T_{FH}) differentiation [17•,18•]. Thus, antagonizing miR-17-92 might be useful in T_{FH}-driven autoimmune disease. These results suggest that pharmacologic miR-17-92 inhibition may preferentially target activated T cells while sparing resting T cells. Ideally this could lead to depletion/inactivation of pathogenic antigen-specific T cells without changing the overall T cell receptor repertoire. However, both effector and regulatory T cells depend on miR-17-92 which makes it unpredictable which way systemic pharmacologic miR-17-92 inhibition would shift the balance. Furthermore, germline deletion of miR-17-92 leads to developmental defects in mice and humans [24,26] but it is unknown if miR-17-92 ablation in the adult or only transient inhibition would also lead to predictable unwanted on-target effects. In that case, cell-type specific delivery of the inhibitor may circumvent the problem.

Preclinical nucleic acid-based therapeutics inhibiting miRNA function

Mature miRNAs can be inhibited by experimental overexpression of miRNA binding sites (miRNA 'sponges') or by pharmacologic administration of antisense oligonucleotides (ASO) [27]. Therefore specific miRNA inhibitors are relatively simple to design. Predictable specificity (Watson-Crick base pairing) of the inhibitors for their miRNA targets is a major advantage over, for example small molecules whose specificity for their target is largely unpredictable (Table 1). Therefore targeting miRNAs with nucleic acid-based drugs promised to cut out medicinal chemistry from the drug development process which should reduce time and costs. Indeed miRNA inhibitors were rapidly developed and used experimentally. A number of chemical modifications improve stability against nucleases, affinity of nucleotide-based miRNA inhibitors and help to overcome the negative charge which inhibits cell penetration [28•]. Independent of their chemical composition we will collectively refer to nucleic acid-based miRNA inhibitors as antimiRs. Although miRNA inhibition can readily be achieved *in vitro*, the biggest challenge to inhibit miRNAs *in vivo* is delivery of antimiRs. To some degree this challenge is addressed by conjugating the antimiRs to carriers or by packaging them into various delivery vehicles [28•,29,30]. Carriers do not only allow or improve cellular uptake *in vivo* but they can also be used to add specificity for target cells and organs. However, guiding specific antimiR delivery to cells of interest by conjugating the carriers to antibodies, ligands or peptides further increases the complexity of the final drug and thus the price. In a promising novel approach the acidic environment of tumors induces a conformational change in the carrier to allow cell permeation of the antimiR. This approach generates selectivity for sites of increased metabolism such as tumors or presumably also sites of inflammation. AntimiR-155 treatment in a miR-155 'addicted' lymphoma model was effective and more selectively targeted the diseased cells than the current standard of care [31••]. Since hypoxia occurs at sites of inflammation this approach will likely also work in miRNA-driven lymphoproliferative and autoimmune settings [16,32].

Another promising class of antimiRs are very short locked nucleic acid (LNA)-modified ASOs targeting miRNA seed regions [33]. LNA seed family inhibitors inhibit entire miRNA families rather than individual miRNAs and even without carriers they have a good bioavailability *in vivo*. LNA-modified antimiR-21 suppressed disease in a lupus mouse model and psoriasis patient-derived skin xenotransplants [34,35••]. These studies are promising because miR-21-deficient mice do not show detectable defects suggesting that there will be limited unwanted side effects. Furthermore, intradermal antimiR-21 delivery to the skin effectively improved disease [35••]. This illustrates that topic administration of antimiRs to readily

Table 1

Comparison of nucleic acid-based drugs and small molecules to target miRNAs

Compound Class	Pro	Contra
Nucleic acid-based	- Prediction of target specificity (Watson/Crick) - Co-evolution of miRNAs and its targets: modulate pathways/genetic networks	- Negatively charged - Instability - Delivery almost exclusively to the liver - Non-specific stimulation of the immune system - Expensive - Little experience: new class of drugs - Currently only parenteral or topic application
Small molecules	- Good tissue penetration - Large libraries are available (5×10^6 compounds) - Quite stable inside the cells - Lots of clinical experience (majority of clinically approved drugs) - Relatively cheap - Oral application	- Unpredictable specificity: Requires high throughput screening followed by medicinal chemistry. (Exception: Informa algorithm allows predictions) [48••]

accessible body surfaces (eyes, skin, oral cavity, airways, vagina, rectum) is a viable alternative approach to overcoming the challenges of systemic administration *in vivo*.

Therapeutically enhancing microRNA function

Depending on the clinical context it may be desirable to pharmacologically enhance miRNA function, for example to substitute lost miRNA function or to repress a miRNA-regulated pathway. For instance the hypoxia-induced miR-210 is a negative regulator of T_H17 differentiation [32]. Therefore delivering miR-210 mimics using the above-mentioned approach to deliver to acidic environments might dampen T_H17-driven inflammation [31••]. Induction of miR-146a is expected to have immunosuppressive effects since it is a negative regulator of several immune cells and enhances regulatory T cell function [9,10,36]. In contrast, transiently inducing miR-17-92 could potentially serve as an adjuvant during immunization since it is induced by CD28-mediated costimulation, is required for several CD28-dependent functions and it is even sufficient to partially compensate for CD28 signaling [16,17•,18•,19•,37]. Induction of miR-17-92 should, however, only be transient since chronic overexpression can drive disease in animal models [16].

Instead of delivering miRNA mimics induction of transcription of endogenous miRNAs could be achieved using CRISPR-based approaches [38]. However, this technology is currently experimental and delivery of the necessary molecules will likely face the same challenges as nucleic acid-based drugs. Another interesting but poorly explored approach is to target RNA structure or miRNA processing. Multi-step miRNA maturation and extensive secondary structures offer opportunities for therapeutic intervention (Figure 1). Targeting the pre-let-7a-2 loop was successfully achieved with a modified oligoribonucleotide termed 'looptomir'. The looptomir was designed to disrupt the inhibitory RBP Lin28 from binding to the pre-let-7a-2 loop which enhanced let-7 processing [39••]. Thus, multiple strategies can be pursued to increase miRNA function.

Successful clinical trials

Nucleic acid-based therapeutics are generally well tolerated in humans and many clinical trials are ongoing [28•,29,40,41]. Short interfering RNAs (siRNAs) are short double stranded RNAs that use the same cellular machinery as miRNAs to achieve gene repression referred to as RNA interference (RNAi). Since siRNAs were explored before miRNAs the siRNA-based drugs tend to be further advanced than miRNA modulating drugs but successful clinical applications of the former should be a good indicator for the latter [28•,29,40]. Mipomersen, a drug to treat a form of familial hypercholesterinemia which targets apolipoprotein B mRNA through an RNAse H-dependent mechanism was recently approved as a systemically administered antisense drug. In addition, a single injection of an siRNA coupled to lipid nanoparticles effectively led to transthyretin (TTR) knockdown in patients with TTR Amyloidosis [42••]. Similarly, miravirsen, an antisense inhibitor of miR-122, resulted in a dose-dependent strong decrease in hepatitis C viral load [43••]. Thus, these important proof-of-principle studies are very encouraging and suggest that drugs targeting RNA work in humans. However, despite major efforts to target multiple organs delivery is most effectively achieved to the liver [28•,29]. In fact, according to publically available information most of the companies specialized on RNAi/miRNA are targeting the liver and none have major programs to treat immune diseases anymore. Therefore, siRNA-based and miRNA modulating drugs are likely to be on the market soon but targeting miRNAs in the immune system might require additional and specific efforts [30].

Alternatives to nucleic acid-based therapeutics

In light of the challenging delivery of nucleic acid-based drugs to immune cells alternative approaches to modulate miRNA function are noteworthy. Small molecules (<500 molecular weight) are the cornerstone of the pharmaceutical industry and overcome the delivery problem (Table 1). A proof-of-principle study demonstrated the successful identification of small molecules that modulate miRNA function with a certain specificity [44]. The cost for ease of delivery is to give up the predictable specificity provided by nucleotide-based therapeutics. Thus, high throughput screenings are required. However, RNA has for a long time been considered 'undruggable' with small molecules despite the fact that successful antibiotics (aminoglycosides) work through direct interaction with RNA. Due to extensive secondary structures RNA is theoretically an attractive target. Possibly our limited understanding of RNA structure and chemotypes that preferentially interact with specific RNA structures may account for this perception [45]. However, screening of a small molecule library against a library of RNA motifs identified chemotypes that preferentially bind RNA which challenges this prevailing view. Moreover, compared to other structures small molecules preferentially bound to RNA hairpin loops which may be relevant to target miRNA biogenesis (Figure 1) [46]. Indeed, based on the known interaction of aminoglycosides with RNA, particularly stem-loops and bulges, Bose *et al.* hypothesized that aminoglycosides could modulate miRNA processing. A small screen of aminoglycosides identified streptomycin as a partially specific miR-21 inhibitor. Mechanistically, streptomycin directly binds to the terminal loop region of pre-miR-21 which blocks Dicer processing (Figure 1) [47]. Thus, miRNA processing can be modulated by direct binding of small molecules to target RNAs. Moreover, a new computational approach called Inforna can predict lead small molecule compounds able to target a RNA of interest [48••]. Predictions were validated experimentally and demonstrated that small molecule miRNA inhibition can be more specific than a nucleic acid-based inhibitor. This powerful algorithm combines available information from an RNA motif-small molecule interaction database with RNA sequence and structure predictions/experimental data and could be extended to any RNA. Thus, Inforna combines the best of two worlds: predictability and ease of delivery although the identified compounds were not very potent [48••].

In summary, small molecules can directly interact with RNA to modulate miRNA processing, achieve remarkable specificity and new approaches even allow predictions of compound/RNA interactions. Since small molecule interactions with RNA are very different from interactions with proteins [49] specific efforts to determine RNA structure [50], RNA binding partners [51] and growing databases of chemotypes interacting with RNA structures might lead to the development of novel small molecule immunomodulatory drugs that target miRNA function.

Conclusions

From the first discovery of a miRNA in a worm more than two decades ago we have come a long way. miRNAs are important regulators of the immune system and constitute attractive pharmacologic targets. Further research will undoubtedly provide a more granular picture of miRNAs and their targets in the immune system as well as immune-mediated diseases. Delivery of nucleic acid-based miRNA modulating drugs is extremely challenging in the immune system but novel and alternative approaches promise that miRNAs will ultimately become drug targets in clinical practice. Future academic and industrial research and development will need to focus on improved *in vivo* delivery strategies. In addition, a better understanding of RNA structure and interactions between small molecules and RNA will pave the way for small molecules to be targeted against RNA. The pharmaceutical industry invested heavily in the pioneering days of RNAi and miRNA research, then faced a phase of disappointment and disinvestments but the recent clinical success stories are sparking the field again and promise to fuel the engine to the finish line of exploiting miRNAs as targets for immunomodulation [52,53].

Conflict of interest statement

Nothing declared.

Acknowledgements

We would like to thank the lab members for discussions and critical comments on the manuscript and the Basel University Hospital and Department of Biomedicine for institutional support. This work was supported by the Swiss National Science Foundation (SNSF Professorship PP00P3_144860) and the National Institute Of Allergy And Infectious Diseases of the National Institutes of Health, USA, under Award Number R56/R01AI106923 and P30 DK063720.

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. Gashaw I, Ellinghaus P, Sommer A, Asadullah K: **What makes a good drug target?** *Drug Discov Today* 2011, **16**:1037-1043.

2. Olive V, Minella AC, He L: **Outside the coding genome, mammalian micrornas confer structural and functional complexity**. *Sci Signal* 2015, **8**:re2.

3. Farh KK, Marson A, Zhu J, Kleinewietfeld M, Housley WJ, Beik S, Shoresh N, Whitton H, Ryan RJ, Shishkin AA, Hatan M *et al.*: **Genetic and epigenetic fine mapping of causal autoimmune disease variants**. *Nature* 2015, **518**:337-343.

4. • Ha M, Kim VN: **Regulation of microrna biogenesis**. *Nat Rev Mol Cell Biol* 2014, **15**:509-524.
Up-to-date detailed review of miRNA biogenesis.

5. • Vidigal JA, Ventura A: **The biological functions of mirnas: lessons from in vivo studies**. *Trends Cell Biol* 2015, **25**:137-147.

Balanced review of how miRNAs function in vivo. Summarizes and clearly explains many important concepts of miRNA biology.

6. Bronevetsky Y, Ansel KM: **Regulation of mirna biogenesis and turnover in the immune system**. *Immunol Rev* 2013, **253**:304-316.

7. Ruegger S, Grosshans H: **Microrna turnover: when, how, and why**. *Trends Biochem Sci* 2012, **37**:436-446.

8. Baumjohann D, Ansel KM: **Microrna-mediated regulation of t helper cell differentiation and plasticity**. *Nat Rev Immunol* 2013, **13**:666-678.

9. Jeker LT, Bluestone JA: **Microrna regulation of t-cell differentiation and function**. *Immunol Rev* 2013, **253**:65-81.

10. Montagner S, Orlandi EM, Merante S, Monticelli S: **The role of mirnas in mast cells and other innate immune cells**. *Immunol Rev* 2013, **253**:12-24.

11. Hu R, O'Connell RM: **Microrna control in the development of systemic autoimmunity**. *Arthritis Res Ther* 2013, **15**:202.

12. Olive V, Sabio E, Bennett MJ, De Jong CS, Biton A, McGann JC, Greaney SK, Sodir NM, Zhou AY, Balakrishnan A, Foth M *et al.*: **A component of the miR-17-92 polycistronic oncomir promotes oncogene-dependent apoptosis**. *eLife* 2013, **2**:e00822.

13. Olive V, Li Q, He L: **Mir-17-92: a polycistronic oncomir with pleiotropic functions**. *Immunol Rev* 2013, **253**:158-166.

14. Simpson LJ, Patel S, Bhakta NR, Choy DF, Brightbill HD, Ren X, Wang Y, Pua HH, Baumjohann D, Montoya MM, Panduro M *et al.*: **A microrna upregulated in asthma airway T cells promotes th2 cytokine production**. *Nat Immunol* 2014, **15**:1162-1170.

15. He L, Thomson JM, Hemann MT, Hernando-Monge E, Mu D, Goodson S, Powers S, Cordon-Cardo C, Lowe SW, Hannon GJ, Hammond SM: **A microrna polycistron as a potential human oncogene**. *Nature* 2005, **435**:828-833.

16. Xiao C, Srinivasan L, Calado DP, Patterson HC, Zhang B, Wang J, Henderson JM, Kutok JL, Rajewsky K: **Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes**. *Nat Immunol* 2008, **9**:405-414.

17. • Baumjohann D, Kageyama R, Clingan JM, Morar MM, Patel S, de Kouchkovsky D, Bannard O, Bluestone JA, Matloubian M, Ansel KM, Jeker LT: **The microrna cluster miR-17-92 promotes T_{FH} cell differentiation and represses subset-inappropriate gene expression**. *Nat Immunol* 2013, **14**:840-848.
These studies identify the importance of miR-17-92 for T_{FH} cell differentiation.

18. • Kang SG, Liu WH, Lu P, Jin HY, Lim HW, Shepherd J, Fremgen D, Verdin E, Oldstone MB, Qi H, Teijaro JR *et al.*: **Microrna of the miR-17-92 family are critical regulators of $T_{(FH)}$ differentiation**. *Nat Immunol* 2013, **14**:849-857.
These studies identify the importance of miR-17-92 for T_{FH} cell differentiation.

19. • de Kouchkovsky D, Esensten JH, Rosenthal WL, Morar MM, Bluestone JA, Jeker LT: **Microrna-17-92 regulates il-10 production by regulatory T cells and control of experimental autoimmune encephalomyelitis**. *J Immunol* 2013, **191**:1594-1605.
Illustration how absence of a miRNA cluster may be dispensable under homeostatic conditions but become essential during an acute inflammatory disease.

20. Chong Yon Park LTJ, Carver-Moore K, Oh A, Jiin Liu H, Cameron R, Richards H, Li Z, Adler D, Yoshinaga Y, Martinez M, Nefadov M, Abbas AK, Weiss A, Lanier LL, de Jong PJ, Bluestone JA, Srivastava D, McManus MT: **A resource for the conditional ablation of micrornas in the mouse**. *Cell Rep* 2012, **1**:1-7.

21. Mendell JT, Olson EN: **Micrornas in stress signaling and human disease**. *Cell* 2012, **148**:1172-1187.

22. Ebert MS, Sharp PA: **Roles for micrornas in conferring robustness to biological processes**. *Cell* 2012, **149**:515-524.

23. Medina PP, Nolde M, Slack FJ: **Oncomir addiction in an in vivo model of microrna-21-induced pre-b-cell lymphoma**. *Nature* 2010, **467**:86-90.

24. Ventura A, Young AG, Winslow MM, Lintault L, Meissner A, Erkeland SJ, Newman J, Bronson RT, Crowley D, Stone JR, Jaenisch R *et al.*: **Targeted deletion reveals essential and overlapping functions of the miR-17-92 family of mirna clusters**. *Cell* 2008, **132**:875-886.

25. Jiang S, Li C, Olive V, Lykken E, Feng F, Sevilla J, Wan Y, He L, Li QJ: **Molecular dissection of the miR-17-92 cluster's critical dual roles in promoting th1 responses and preventing inducible treg differentiation**. *Blood* 2011, **118**:5487-5497.

26. de Pontual L, Yao E, Callier P, Faivre L, Drouin V, Cariou S, Van Haeringen A, Genevieve D, Goldenberg A, Oufadem M, Manouvrier S *et al.*: **Germline deletion of the miR-17-92 cluster causes skeletal and growth defects in humans**. *Nat Genet* 2011, **43**:1026-1030.

27. Broderick JA, Zamore PD: **Microrna therapeutics**. *Gene Ther* 2011, **18**:1104-1110.

28. • Li Z, Rana TM: **Therapeutic targeting of micrornas: current status and future challenges**. *Nat Rev Drug Discov* 2014, **13**:622-638.
Very balanced and comprehensive review about exploiting miRNAs therapeutically. Includes chemical modifications of nucleic acid-based drugs, delivery vehicles/approaches and tables of (pre)clinical developments of RNAi and miRNA-based drugs.

29. Kanasty R, Dorkin JR, Vegas A, Anderson D: **Delivery materials for sirna therapeutics**. *Nat Mater* 2013, **12**:967-977.

30. Peer D: **A daunting task: manipulating leukocyte function with rnai**. *Immunol Rev* 2013, **253**:185-197.

31. •• Cheng CJ, Bahal R, Babar IA, Pincus Z, Barrera F, Liu C, Svoronos A, Braddock DT, Glazer PM, Engelman DM, Saltzman WM *et al.*: **Microrna silencing for cancer therapy targeted to the tumour microenvironment**. *Nature* 2015, **518**:107-110.
An innovative new approach to circumvent in vivo delivery obstacles which are a main bottleneck for the development of miRNA targeting drugs. A carrier that changes its conformation in acidic microenvironments promises to deliver its cargo (miRNA inhibitor or mimic) to sites of disease (tumors but likely also inflammatory sites).

32. Wang H, Flach H, Onizawa M, Wei L, McManus MT, Weiss A: **Negative regulation of hif1a expression and th17 differentiation by the hypoxia-regulated microrna mir-210**. *Nat Immunol* 2014, **15**:393-401.

33. Obad S, dos Santos CO, Petri A, Heidenblad M, Broom O, Ruse C, Fu C, Lindow M, Stenvang J, Straarup EM, Hansen HF *et al.*: **Silencing of microrna families by seed-targeting tiny lnas**. *Nat Genet* 2011, **43**:371-378.

34. Garchow BG, Bartulos Encinas O, Leung YT, Tsao PY, Eisenberg RA, Caricchio R, Obad S, Petri A, Kauppinen S, Kiriakidou M: **Silencing of microrna-21 in vivo ameliorates autoimmune splenomegaly in lupus mice**. *EMBO Mol Med* 2011, **3**:605-615.

35. •• Guinea-Viniegra J, Jimenez M, Schonthaler HB, Navarro R, Delgado Y, Concha-Garzon MJ, Tschachler E, Obad S, Dauden E, Wagner EF: **Targeting mir-21 to treat psoriasis**. *Sci Transl Med* 2014, **6**:225re221.
Using sophisticated psoriasis-patient derived human xenotransplants in mice the authors validate miR-21 as a valid target in psoriasis. Intradermal antimiR-21 injections (LNA-modified seed inhibitors) significantly improved disease.

36. Hu R, Kagele DA, Huffaker TB, Runtsch MC, Alexander M, Liu J, Bake E, Su W, Williams MA, Rao DS, Moller T *et al.*: **Mir-155 promotes t follicular helper cell accumulation during chronic, low-grade inflammation**. *Immunity* 2014, **41**:605-619.

37. Wang CJ, Heuts F, Ovcinnikovs V, Wardzinski L, Bowers C, Schmidt EM, Kogimtzis A, Kenefeck R, Sansom DM, Walker LS: **Ctla-4 controls follicular helper t-cell differentiation by regulating the strength of cd28 engagement**. *Proc Natl Acad Sci U S A* 2015, **112**:524-529.

38. Konermann S, Brigham MD, Trevino AE, Joung J, Abudayyeh OO, Barcena C, Hsu PD, Habib N, Gootenberg JS, Nishimasu H, Nureki O *et al.*: **Genome-scale transcriptional activation by an engineered crispr-cas9 complex**. *Nature* 2015, **517**:583-588.

39. •• Roos M, Rebhan MA, Lucic M, Pavlicek D, Pradere U, Towbin H, Civenni G, Catapano CV, Hall J: **Short loop-targeting oligoribonucleotides antagonize lin28 and enable pre-let-7 processing and suppression of cell growth in let-7-deficient cancer cells**. *Nucleic Acids Res* 2015, **43**:e9.
Innovative approach to increase/restore miRNA expression through targeted antagonization of an inhibitory pre-miR processing RNA binding protein.

40. Lightfoot HL, Hall J: **Target mrna inhibition by oligonucleotide drugs in man**. *Nucleic Acids Res* 2012, **40**:10585-10595.

41. Wu SY, Lopez-Berestein G, Calin GA, Sood AK: **Rnai therapies: drugging the undruggable**. *Sci Transl Med* 2014, **6**:240ps247.

42. •• Coelho T, Adams D, Silva A, Lozeron P, Hawkins PN, Mant T, Perez J, Chiesa J, Warrington S, Tranter E, Munisamy M *et al.*: **Safety and efficacy of rnai therapy for transthyretin amyloidosis**. *N Engl J Med* 2013, **369**:819-829.
Two important clinical studies demonstrating that RNAi and targeting miRNAs in humans can be safe and efficacious.

43. •• Janssen HL, Reesink HW, Lawitz EJ, Zeuzem S, Rodriguez-Torres M, Patel K, van der Meer AJ, Patick AK, Chen A, Zhou Y, Persson R *et al.*: **Treatment of hcv infection by targeting microrna**. *N Engl J Med* 2013, **368**:1685-1694.
Two important clinical studies demonstrating that RNAi and targeting miRNAs in humans can be safe and efficacious.

44. Gumireddy K, Young DD, Xiong X, Hogenesch JB, Huang Q, Deiters A: **Small-molecule inhibitors of microrna mir-21 function**. *Angew Chem Int Ed Engl* 2008, **47**:7482-7484.

45. Velagapudi SP, Vummidi BR, Disney MD: **Small molecule chemical probes of microrna function**. *Curr Opin Chem Biol* 2015, **24**:97-103.

46. Tran T, Disney MD: **Identifying the preferred rna motifs and chemotypes that interact by probing millions of combinations**. *Nat Commun* 2012, **3**:1125.

47. Bose D, Jayaraj G, Suryawanshi H, Agarwala P, Pore SK, Banerjee R, Maiti S: **The tuberculosis drug streptomycin as a potential cancer therapeutic: Inhibition of mir-21 function by directly targeting its precursor**. *Angew Chem Int Ed Engl* 2012, **51**:1019-1023.

48. •• Velagapudi SP, Gallo SM, Disney MD: **Sequence-based design of bioactive small molecules that target precursor micrornas**. *Nat Chem Biol* 2014, **10**:291-297.
This paper describes a new computational approach to predict small molecule lead compounds that interact with RNA structure solely based on RNA sequence information. Thus, this study suggests that the benefits of using small molecules could be combined with predictability.

49. Disney MD, Yildirim I, Childs-Disney JL: **Methods to enable the design of bioactive small molecules targeting rna**. *Org Biomol Chem* 2014, **12**:1029-1039.

50. Spitale RC, Flynn RA, Zhang QC, Crisalli P, Lee B, Jung JW, Kuchelmeister HY, Batista PJ, Torre EA, Kool ET, Chang HY: **Structural imprints in vivo decode rna regulatory mechanisms**. *Nature* 2015, **519**:486-490.

51. Imig J, Brunschweiger A, Brummer A, Guennewig B, Mittal N, Kishore S, Tsikrika P, Gerber AP, Zavolan M, Hall J: **Mir-clip capture of a mirna targetome uncovers a lincrna h19-mir-106a interaction**. *Nat Chem Biol* 2015, **11**:107-114.

52. Haussecker D: **The business of rnai therapeutics in 2012**. *Mol Ther Nucleic Acids* 2012, **1**:e8.

53. Haussecker D, Kay MA: **Rna interference. Drugging rnai**. *Science* 2015, **347**:1069-1070.