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CONTENT

Prognostic significance of GATA4 in adult type granulosa cell tumor of the ovary

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ABSTRACT

Background: Adult type granulosa cell tumor is the most common sex cord-stromal tumor. It constitutes approximately 1% of all ovarian tumors. Granulosa cell tumors have a slow course, most patients are diagnosed at an early stage, but the recurrence rate is high. *GATA4* is a "zinc-finger" transcription factor that plays an important role in granulosa cell function and development. Overexpression of GATA4 protects cells from apoptosis. **Aims:** The aim of the study was to evaluate the level of GATA4 expression in adult-type granulosa cell tumors, to determine the relationship of this expression with clinicopathological features and to reveal its effect on survival. **Settings and Design**: GATA4 expression level was determined immunohistochemically. **Material and Methods**: Cases diagnosed with granulosa cell tumor in our clinic between 2010 and 2020 were evaluated retrospectively. Paraffin blocks that best reflected the morphology were selected. **Statistical Analysis Used**: Fisher exact test, Pearson $\chi 2$ test, Kaplan-Meier method, Cox regression test were used. **Results:** GATA4 expression level was found to be higher in early stage cases. GATA4 expression level was not associated with overall survival or disease-free survival. **Conclusion:** Higher expression in early stage cases indicates that GATA4 supports good prognostic features. Expression level does not affect survival time. It is necessary to reveal the parameters that can be used to predict prognosis and recurrence. GATA4 may be a potential marker here.

Key words: Adult type granulosa cell tumor, GATA4, Ovarian neoplasm, Prognosis

INTRODUCTION

Of the sex cord-stromal tumors 90% are granulosa cell tumors. There are two subtypes of these tumors. Adult and juvenile type. Adult-type granulosa cell tumor (AGCT) consists of granulosa cells growing in a variety of patterns. Juvenile granulosa cell tumor (JGCT) is a sex cord tumor composed of primitive-appearing cells growing in solid and follicular patterns. AGCT accounts for approximately 95% of all granulosa cell tumors (Pectasides et al, 2008:34, Stuart and Dawson, 2003:15). These tumors are mostly seen in the perimenopausal period. The most common causes of symptoms that patients present with include estrogenic symptoms such as menometrorrhagia or postmenopausal bleeding. A proportion of patients may have concomitant endometrial hyperplasia or endometrioid carcinoma (WHO Classification of Tumours Editorial Board. Female genital tumours, 2020, 5th ed.). AGCT may rarely present with androgenic symptoms (Nakashima N, 1984:108). Tumors are mostly unilateral. Different growth patterns can be seen within the same tumor on microscopic examination. The nuclei of tumor cells are oval or round. Contains pale chromatin and nuclear

groove. The cytoplasm is narrowand the mitotic rate usually does not exceed 5 in 10 high power fields (WHO Classification of Tumours Editorial Board. Female genital tumours, 2020, 5th ed.).

AGCT has a low biological malignancy potential. Most patients are diagnosed at an early stage. Relapses or distant metastases occur long after diagnosis. Death occurs in 50-80% of patients with recurrence (Bryk et al, 2015:25, McConechy, 2016:108). Management of patients is difficult because of the late recurrence. Cellular mechanisms of latent period and late relapse could not be determined, but tumor dormancy mechanisms similar to those reported in breast cancer have been suggested (Färkkilä et al, 2017:49).

Parameters such as age, tumor size, clinical stage, extent of surgery, presence of residual disease, and tumor rupture have been suggested as clinical prognostic factors (WHO Classification of Tumours Editorial Board. Female genital tumours, 2020, 5th ed, Färkkilä et al, 2017:49, Sakr et al, 2017:36). Among histopathological factors, features such as tumor growth pattern, mitotic index, nuclear atypia, and inhibin, epidermal growth factor, and Ki67 expression have been examined (Sakr et al, 2017:36). No parameter has been found to reliably predict recurrence. FIGO stage is the only consistent prognostic factor associated with survival (Bryk et al, 2015:25, Sun et al, 2012:124, Lee et al, 2008:18). The transcription factor *FOXL2* is one of the early markers of ovarian differentiation. It is required for the normal development of granulosa cells and has an important role in regulating follicular formation (Cocquet et al, 2002:39). It is also involved in the regulation of apoptosis, oxidative stress response and cell proliferation processes. Recurrent missense somatic mutation of *FOXL2* was described in AGCT (Shah et al, 2009:360). *FOXL2* mutations can also be seen in thecomas, juvenile granulosa cell tumors and fibromas (Shah et al, 2009:360, Kim, 2010:221). Tumors with homozygous *FOXL2* mutations have been reported to have higher recurrence rates (D'Angelo et al, 2011;24).

GATA4 is a member of the "zinc-finger" family of transcription factors. The role of *GATA4* is important in the development of tissues and surface epithelium (Li, 2018:11). It regulates cell apoptosis and proliferation via Bcl-2 and cyclin D2 (Kyronlahti et al, 2008:149). Overexpression of GATA4 has been suggested to be associated with frequent recurrence and aggressive biological behavior in granulosa cell tumors (Anttonen 2005:90). Conversely, there are studies suggesting that GATA4 suppresses tumor growth (Fang, 2009:157). In addition, there are publications suggesting that there is no prognostic relationship between GATA4 and granulosa cell tumors (Levin G, 2018:225).

It is necessary to reveal the factors affecting recurrence and survival in granulosa cell tumors. Longer disease-free survival can be achieved by predicting the recurrence of patients. The aim of the study was to evaluate the level of GATA4 expression in adult-type granulosa cell tumors, to

determine its relationship with clinicopathological features and to investigate its effect on recurrence and survival.

MATERIAL AND METHODS

Patients and clinical parameters: The cases diagnosed with granulosa cell tumors in our department between 2010 and 2020 were evaluated retrospectively. Parameters such as age, tumor size, lymph node status, number of mitoses (in 10 high power fields) of the tumor were obtained from the pathology records. Features such as clinical manifestations, clinical stage, treatment method, recurrence status and survival time were obtained from the gynecological oncology clinic. FIGO classification (2021 FIGO Cancer Report) was used for staging.

Immunohistochemistry: Paraffin blocks were available and areas with tumor elements were selected on H&E slides and marked on the corresponding paraffin blocks. Paraffin blocks were cut into 4 μm thick sections and slides were deparaffinized. Reticulin staining was completed for missing cases. FOXL2 (Medaysis, clone: RC0107) and GATA4 (Medaysis, clone: MC0169) immunohistochemical studies were performed with an automated immunohistochemistry staining device (Dako Omnis Automated IHC/ISH Staining System) according to the manufacturer's instructions. DAB (diaminobenzidine) was used as the chromogen for signal detection and cells were counterstained with Harris hematoxylin. Negative controls were incubated with the same concentration of immunoglobulin (IgG1; Dako, Ely, UK) instead of the primary antibody. The positive control was ovarian tissue. The percentage and intensity of staining with GATA4 and the intensity of staining with FOXL2 were investigated.

Statistical analysis: The critical value of GATA4 staining percentage was detected by ROC (receiver operating characteristic) curve. Staining percentage was classified as “high” for those above the critical value and “low” for those below the critical value. Nuclear staining was considered positive for both markers. Staining intensity was grouped as “weak”, “medium” and “strong” for GATA4, and “weak” and “strong” for FOXL2. Statistically significant relationships were investigated between staining results and clinicopathological features, recurrence status and survival time. SPSS version 21.0 software was used for statistical analysis. Fisher exact test was performed for any 2×2 table, Pearson $\chi 2$ test for non-2×2 tables and $\chi 2$ trend test for ordinal data. Survival analysis was performed using the Kaplan-Meier method. Univariate and multivariate analyses were evaluated using the Cox regression test. P values <0.05 were considered significant.

RESULTS

There were 89 patients in total. Of these, 9 patients whose follow-up data could not be reached were not included. As a result of the slides review, 5 cases were evaluated as juvenile type granulosa cell tumor, 1 case as Sertoli cell tumor, 4 cases as fibroma and 1 case as Brenner tumor, so they were excluded from the study. The remaining 69 cases were included in the study. The mean age of the patients was 53.26 (30-83). The average body mass index of the cases was 30,4± 5,9.The most common complaint of the patients was abnormal vaginal bleeding (52.2%). Pain (34.8%) and mass sensation (13%) were other reasons for admission. The tumor was located in the left ovary in 53.6% of the cases, in the right ovary in 33.3%, and bilaterally in 13.1%. The mean tumor size was 8.86 cm (1-27 cm) (Table 1).

According to FIGO staging, 32 cases (46.4%) were stage Ia, 28 (40.6%) were stage Ic, 2 (2.9%) were stage II, 2 (2.9%) were stage III and 5 were (7.2%) stage IV. Recurrence developed in 9 cases after treatment. Seven cases died due to tumor-related causes. Mean disease-free survival time was 48.9 months (between 3-132 months) and overall survival was 70.8 months (between 3-366 months). Clinicopathological features of the cases are given in Table 1.Reticular fiber was decreased histochemically in all of the cases.

Table 1. Clinicopathological features of the cases

		n	%
Age	average (range; years)	53,26 (30-83)	
Tumor size	average (range; cm)	8,86 (1-27)	
Number of mitosis in 10 high power field	average (range)	9,22 ± 18,8 (1-100)	
Body-mass index	average (range)	30,4 ± 5,9 (20-44)	
Laterality	right ovary	23	33,3
	left ovary	37	53,6
	bilateral	9	13,1
Complaint	abnormal bleeding	36	52,2
	pain	24	34,8
	mass sensation	9	13
Menopause status	no	24	34,8
	yes	45	65,2
Clinical stage	Ia	32	46,4
	Ic	28	40,6
	II	2	2,9
	III	2	2,9
	IV	5	7,2
Surgical treatment	limited surgery	24	34,8
	radical surgery	45	65,2
Reduction in reticular fibers	slight	19	27,5
	evident	50	72,5
FOXL2 staining	negative	14	20,3
	weak positive	48	69,6

	strongly positive	7	10,1
Recurrence	(-)	60	87
	(+)	9	13
Disease-related death	(-)	62	89,9
	(+)	7	10,1

FOXL2 was negative in 14 (20.3%) cases. FOXL2 staining was not statistically correlated with clinicopathological parameters or survival. Those with GATA4 staining percentage ≥82.5% were considered as "high", and with <82.5% staining were considered as "low" staining percentage. High staining percentage was found in approximately 2/3 (68.8%) of the cases, and low staining percentage was found in 1/3. Clinicopathological parameters did not differ significantly in cases with low and high staining percentages. When the staining intensity was evaluated, strong staining was found in 36.2% (25 cases), moderate staining in 36.2% (25 cases), and weak staining in 24.6% (17 cases) (shown in Fig. 1). GATA4 was negative in 2 (2.9%) cases. There was a statistically significant correlation between GATA4 staining intensity and clinical stage (p=0.002). Accordingly, the intensity of GATA4 staining was significantly higher in early-stage cases than in advanced-stage cases. A stronger GATA4 staining intensity was detected in patients with pain than in those with bleeding and swelling complaints (p=0.026). No significant correlation was found between GATA4 staining intensity and FOXL2 staining status, reticular fiber density, mitotic number, gravida, partus, menopausal status, body-mass index, treatment modality, tumor size, tumor lateralization, or endometrial status (shown in table 2). In the survival analysis, the percentage of GATA4 staining and the intensity of the staining had no effect on disease-free survival (p=0.321 and p=0.545, respectively). Similarly, the percentage and intensity of staining did not affect overall survival (p=0.201 and p=0.616, respectively). Of the clinicopathological parameters, only FIGO stage was found to affect survival (shown in table 3). In early stage (stage I) cases, overall survival time was significantly higher than in other stages (p=0.002). No significant relationship was found between stage and disease-free survival (p=0.298) (Shown in Fig. 2). When the number of mitosis was evaluated, the mean number of mitosis was significantly higher in the patients who lost their lives compared to those who were still alive (mean 28.5 ± 47,7 vs 5.9 ± 5,2 mitosis/10 high power field (HPF), p<0.001). The mean number of mitosis was also statistically different in cases with and without recurrence (mean 37.7 ± 54 vs 5.4 ± 5,1 mitoses/10 HPF, p=0.007).

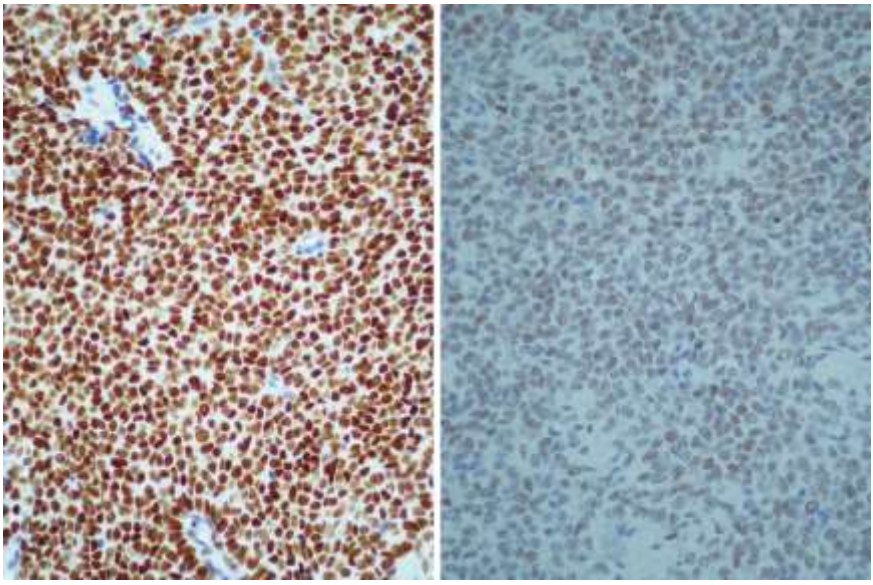

Figure 1: Immunohistochemically strong nuclear GATA4 positivity (left) and weak nuclear staining (right) (MC0169, x400).

Table 2. GATA4 staining intensity and clinicopathological parameters

		Negative n (%)	Weak positive n (%)	Moderate positive n (%)	Strong positive n (%)	P value
Age	≤54	1 (2,6)	11 (28,9)	12 (31,7)	14 (36,8)	0,801
	>54	1 (3,2)	6 (19,4)	13 (41,9)	11 (35,5)	
Laterality	right ovary	0 (0)	6 (26,1)	12 (52,2)	5 (21,7)	0,231
	left ovary	1 (2,7)	8 (21,6)	11 (29,7)	17 (45,9)	
	bilateral	1 (11,1)	3 (33,3)	2 (22,2)	3 (33,3)	
Complaint	abnormal bleeding	0 (0)	6 (16,7)	14 (38,9)	16 (44,4)	**0,026**
	pain	2 (8,3)	9 (37,5)	10 (41,7)	3 (12,5)	
	mass sensation	0 (0)	2 (22,2)	1 (11,1)	6 (66,7)	
Menopause status	no	0 (0)	6 (25)	7 (29,2)	11 (45,8)	0,470
	yes	2 (4,4)	11 (24,4)	18 (40)	14 (31,2)	
Body-mass index	<30	1 (2,8)	9 (25,8)	11 (31,4)	14 (40)	0,869
	≥30	1 (2,9)	8 (23,5)	14 (41,2)	11 (32,4)	
Tumor size	≤8	0 (0)	9 (23,7)	17 (44,7)	12 (31,6)	0,201
	>8	2 (6,5)	8 (25,8)	8 (25,8)	13 (41,9)	
Number of mitosis	≤5	1 (2,4)	5 (12,2)	17 (41,5)	18 (43,9)	0,762
	>5	1 (3,6)	7 (25)	10 (35,8)	10 (35,8)	
Clinical stage	Ia	0 (0)	6 (18,8)	15 (46,9)	11 (34,4)	**0,002**
	Ic	0 (0)	5 (17,9)	9 (32,1)	14 (50)	
	II	0 (0)	2 (100)	0 (0)	0 (0)	
	III	1 (50)	1 (50)	0 (0)	0 (0)	
	IV	1 (20)	3 (60)	1 (20)	0 (0)	
Surgical treatment	limited surgery	1 (2,2)	12 (26,7)	17 (37,8)	15 (33,3)	0,912

	radical surgery	1 (4,2)	5 (20,8)	8 (33,3)	10 (41,7)	
Reduction in reticular fibers	slight	0 (0)	6 (31,6)	9 (47,4)	4 (21,2)	0,280
	evident	2 (4)	11 (22)	16 (32)	21 (42)	
FOXL2 staining	negative	1 (7,7)	3 (23,1)	5 (38,5)	4 (30,7)	0,562
	weak positive	1 (2,1)	11 (22,9)	17 (35,4)	19 (39,6)	
	strongly positive	0 (0)	3 (37,5)	2 (25)	3 (37,5)	
Recurrence	(-)	2 (3,3)	13 (21,7)	23 (38,2)	22 (36,7)	0,472
	(+)	0 (0)	4 (44,4)	2 (22,2)	3 (33,3)	
Disease-related death	(-)	2 (3,2)	15 (24,2)	23 (37,1)	22 (35,5)	0,920
	(+)	0 (0)	2 (28,6)	2 (28,6)	3 (42,9)	

Bold texts are statistically significant p values

Table 3. Analysis of univariate survival in granulosa cell tumor

	Overall survival P value	**Disease-free survival P value**
FIGO stage	**= 0,002**	**< 0,001**
GATA4 staining percentage	= 0,201	= 0,321
GATA4 staining intensity	= 0,616	= 0,167
FOXL2 staining intensity	= 0,486	= 0,247
Density of reticular fibers	= 0,346	= 0,139
Number of mitosis	= 0,846	= 0,480
Menopause status	= 0,782	= 0,420
Body-mass index (<30 vs. ≥30)	= 0,122	= 0,240
Complaint	= 0,776	= 0,277
Tumor size (≤8 vs >8 cm)	= 0,312	= 0,135

Bold texts are statistically significant p values

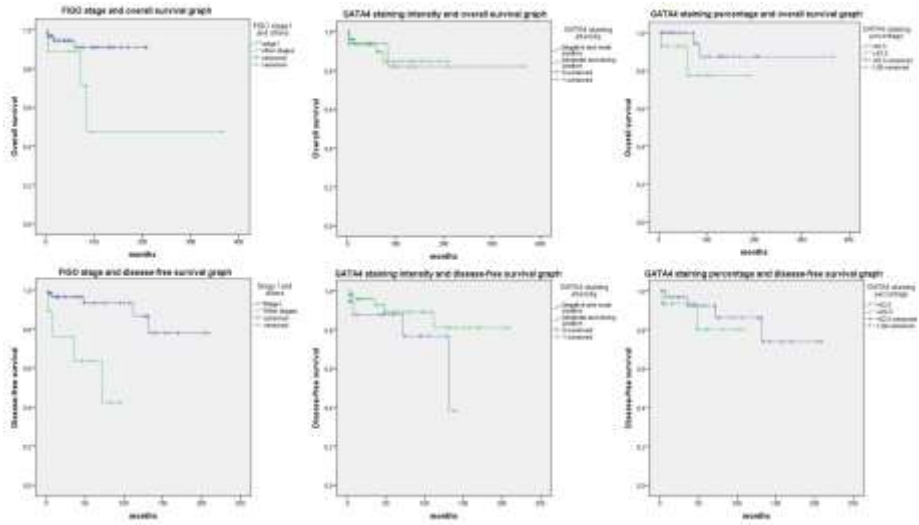

Figure 2: Analysis plots of overall and disease-free survival by FIGO stage, GATA4 staining intensity and percentage of staining.

DISCUSSION

AGCTs are tumors that can have hormonal activities and show clinically different characteristics due to estrogen and inhibin production (WHO Classification of Tumours Editorial Board. Female genital tumours, 2020, 5th ed.). The complaints are usually abnormal vaginal bleeding, abdominal pain or mass sensation (Bryk et al, 2015:25). Spontaneous rupture of the tumor results in acute abdomen and hemoperitoneum in 8-15% of cases. Endometrial pathologies may also accompany the tumor. In microscopic examination, 34-45% synchronous endometrial pathology such as hyperplasia or carcinoma has been reported(Bryk et al, 2015:25). In our study, abnormal bleeding was the most common complaint too. Concomitant endometrial pathology was observed in 44.4% (8.3% endometrial hyperplasia with atypia, 36.1% endometrioid carcinoma) of the cases. AGCTs do not show as aggressive biological behavior as epithelial malignancies of the ovary. Overall survival rates at 5, 10, and 15 years in the 103 patients analyzed were 92.6%, 91%, and 87.4%, respectively (Hauspy 2011:79). The median time of recurrence is 7.2 years (McConechy et al, 2016:108). Levin et al. reported the mean operative-recurrence interval in AGCT as 48-57 months. The recurrence rate varies between 10-64% (2018:225). However, these tumors may recur even 30-40 years after diagnosis (WHO Classification of Tumours Editorial Board. Female genital tumours, 2020, 5th ed.). In our study the average recurrence time was 48.9 months. Late onset of recurrence makes it difficult to determine the treatment options. The cellular mechanisms of late relapse in AGCTs have yet to be determined. Although prognostic indicators have been investigated by many studies, it has not been possible to predict the recurrence probability clearly. More reliable clinical and pathological markers are needed to predict the biological behavior of AGCT.

Age, tumor size, stage of disease, extent of surgery, and residual disease and tumor rupture were investigated as clinical prognostic factors (WHO Classification of Tumours Editorial Board. Female genital tumours, 2020, 5th ed, Färkkilä, 2017:49, Sakr, 2017:36). While some studies indicate that advanced age, large tumor size (>10 cm or >15 cm) and tumor rupture are associated with poor prognosis, other studies have not proven this relationship (Sakr, 2017:36). Another clinical factor that has been suggested to be associated with relapse is a higher body mass index (van Meurs, 2015:94). Presence of residual disease after surgery (Sun, 2012:124), fertility-sparing surgery and adjuvant chemotherapy after surgery (Meisel et al, 2015:136) are treatment-related risk factors for recurrence. In our study, the relationship between these factors and recurrence or prognosis could not be demonstrated. Tumor stage is an independent prognostic factor associated with disease-free and overall survival (Bryk et al, 2015:25, Sun, 2012:124,

Lee, 2008:18). In our study, a significant correlation was found between tumor stage and prognosis too (p=0.002).

Among histopathological factors, tumor histology, mitotic index, nuclear atypical inhibin, epidermal growth factor (EGFR) and Ki67 expression level are recently investigated potential prognostic features.[5,8,9] Although there are studies suggesting that high mitotic activity and nuclear atypia are poor prognostic factors (Sakr, 2017:36) there are also publications that fail to demonstrate this relationship (WHO Classification of Tumours Editorial Board. Female genital tumours, 2020, 5th ed, Färkkilä, 2017:49). Microscopic growth pattern is not associated with prognosis. HER2, CD56 and SMAD3 expression have been suggested as histological features associated with recurrence (Sakr, 2017:36). Suri et al. showed by multivariate analysis that the strongest predictor for relapse was the presence of diabetes mellitus (2013:131). In our study, the effect of clinicopathological factors on prognosis could not be demonstrated except the clinical stage. Although no effect on survival could be demonstrated, average number of mitoses was found to be significantly higher in deceased patients than in those still alive (mean 28.5 vs 5.9 mitoses/10 high magnification field, p<0.001). Mitotic index was also significantly different in cases with and without recurrence (mean 37.7 vs 5.4 mitoses/10 high power field, p=0.007).

It is important to identify risk factors in early-stage cases, as most patients are stage I. Even in early-stage disease, recurrence rates of 20-30% have been reported (WHO Classification of Tumours Editorial Board. Female genital tumours, 2020, 5th ed.). Relapse progresses to death in 50–80% of patients (Bryk et al, 2015:25, McConechy et al, 2016:108). Recurrence was observed in 9 (13%) of the cases in our cohort, and disease-related death occurred in 7 of them.

FOXL2 plays an important role in follicular development in the normal ovary. This is necessary for granulosa cell differentiation (Uhlenhaut et al, 2009;139). On the other hand, the role of *FOXL2* in the mechanism of tumorigenesis in AGCTs is an active area of research. *FOXL2* inhibits cell cycle progression and promotes apoptosis. In contrast, the C134W mutant form activates genes involved in cell proliferation and inhibits apoptosis (Kim et al, 2011;30). The role of the *FOXL2* mutation in the carcinogenesis of granulosa cell tumors has been demonstrated, but few studies have examined the prognostic significance of its immunohistochemical expression level. The results in these studies are also inconsistent. Both decreased and increased expression have been defined as poor prognostic factors (Kalfa et al, 2007:87, Rosario et al, 2013:131). In our study, no relationship was found between FOXL2 expression and clinicopathological parameters or survival. As the number of studies increases, the prognostic importance of FOXL2 will be clearly demonstrated.

The GATA family of proteins play an important role in epithelial proliferation and development. Initially, GATA1, 2 and 3 were defined as hematopoietic factors, and GATA4, 5 and 6 as endodermal factors (Lentjes, 2016:18). GATA1 and 2 are involved in the regulation of cell cycle and proliferation. GATA3 is both an important transcription factor for T cell development and is involved in the proliferation and differentiation of luminal epithelial and urothelial epithelial cells (Levin, 2018:225). GATA4 and 5 are expressed in differentiated epithelial cells and play a tumor suppressor role. GATA6 is found in immature cells in intestinal crypts and is a potential oncogene (Maeda, 2005:47). GATA expression plays a critical role in carcinogenesis in various cancers (Zhou, 2022:15).

GATA4 plays an important role in granulosa cell development. In granulosa cells, follicle-stimulating hormone (FSH) and TGF-β increase the expression of GATA4. GATA4 expression correlates with Bcl 2 and cyclin D2 expression. High expression of GATA4 protects tumor cells from apoptosis (Kyronlahti, 2010:17). GATA4 physically interacts with SMAD3 and FOXL2, affecting cell viability and apoptosis in AGCTs (Anttonen, 2014:9). These data suggest that GATA4 acts as an anti-apoptotic agent in AGCTs. Its high expression is associated with advanced stage and risk of recurrence (Anttonen, 2005:90). In contrary, Patel et al. reported that high expression of GATA4 suppressed tumor growth in non-immunosuppressed mice. GATA4 can perform its tumor suppressor function with the activation of TIL (tumor infiltrating lymphocytes) (Patel, 2022:13). GATA4 exerts its functions independent of the TP53-CDKN1A and RB-CDKN2A pathways. It also has an activating effect on the NFkB transcription factor (Kang, 2015:349). It has been stated that *GATA4* may be a tumor suppressor gene in glioblastoma, ovarian, endometrial and colorectal carcinomas (Hellebrekers, 2009:15). Although studies initially mentioned the tumor supporting role of GATA4, it has been stated in recent publications that it has a tumor suppressive effect. In our study, GATA4 expression was found to be higher in early stage cases. Although no significant relationship could be demonstrated between survival times and GATA4 expression, our results support that high expression level indicates good prognostic features. As the complicated steps related to cell growth and tumor progression are revealed and included in studies, the effects of molecules such as GATA4 will be revealed more clearly.

Preventing recurrence in AGCTs will significantly improve patient survival. The clinical applicability of the parameters claimed to be associated with prognosis is unclear. GATA4 emerges as a potential marker at this stage. More comprehensive studies are needed to make these parameters reliably usable in predicting recurrence and prognosis, and in making treatment decisions.

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Contributors: Deniz Arık, Işıl Bağcı conceptualized the study and provided cases. Elçin Telli, Tufan Öge, Ömer Tarık Yalçın contributed cases. Deniz Arık, Işıl Bağcı performed immunohistochemical evaluation. Deniz Arık, Işıl Bağcı, Elçin Telli analyzed the data. Deniz Arık drafted the manuscript, and all authors were involved with editing the final manuscript. Deniz Arık accept responsibility for the overall content/data.

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Current Therapies in Age-Related Macular Degeneration

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ABSTRACT

Age-related macular degeneration (AMD) remains a leading cause of irreversible central vision loss among the elderly worldwide. The therapeutic landscape has evolved substantially over the past two decades, transitioning from supportive care to targeted, disease-modifying interventions. In neovascular AMD, intravitreal anti–vascular endothelial growth factor (anti-VEGF) therapy continues to represent the cornerstone of management, with robust evidence supporting long-term visual stabilization and anatomical improvement. Recent advances, including dual-pathway inhibitors and extended-interval regimens, aim to reduce treatment burden while maintaining efficacy. In geographic atrophy (GA), complement inhibition has emerged as a novel therapeutic strategy. Avacincaptad pegol, a complement C5 inhibitor, has demonstrated a significant reduction in lesion growth rates in phase III clinical trials, marking a milestone in non-neovascular AMD treatment. Nevertheless, careful monitoring for conversion to neovascular disease remains essential. Emerging approaches such as gene therapy seek to provide sustained intraocular production of anti-angiogenic proteins, potentially minimizing injection frequency. Additionally, lifestyle modification, nutritional supplementation, and systemic risk factor optimization remain integral components of comprehensive care. Collectively, current AMD management reflects a paradigm shift toward personalized, biomarker-guided, and multimodal therapeutic strategies aimed at preserving visual function and improving long-term outcomes.

Keywords: Age-related macular degeneration, Anti-VEGF therapy, Geographic atrophy, Complement inhibition, Gene therapy

1. Introduction

Age-related macular degeneration (AMD) is a multifactorial, progressive retinal disorder and a leading cause of irreversible central vision loss in individuals over 60 years of age. The disease spectrum ranges from early and intermediate stages characterized by drusen and pigmentary abnormalities to advanced forms, namely neovascular (exudative) AMD and geographic atrophy (GA). The socioeconomic burden of AMD continues to rise in parallel with global population aging. Over the past two decades, therapeutic innovation has reshaped the management of neovascular AMD through intravitreal anti–vascular endothelial growth factor (anti-VEGF) therapy. More recently, the introduction of complement inhibition for GA has marked a paradigm shift toward disease-modifying treatment in non-neovascular AMD. In addition, gene-based therapies, long-acting biologics, biomarker-

guided treatment algorithms, and systemic risk factor modification strategies are increasingly integrated into modern AMD care. This chapter reviews current and emerging therapeutic approaches with emphasis on efficacy, safety, and translational implications.

2. Neovascular AMD

2.1 Anti-VEGF Therapy: Foundation of Modern Management

Intravitreal anti-VEGF agents remain the gold standard for neovascular AMD. These agents inhibit VEGF-mediated angiogenesis, vascular permeability, and inflammatory signaling, thereby reducing intraretinal and subretinal fluid accumulation. Long-term data have demonstrated that sustained treatment can preserve vision in a significant proportion of patients. However, cumulative treatment burden and safety considerations remain central issues. A comprehensive safety review of approved neovascular AMD therapies highlighted intraocular inflammation, rare occlusive vasculitis events, and systemic vascular concerns as areas requiring ongoing surveillance [1]. Although serious adverse events are uncommon, prolonged exposure over years necessitates individualized risk assessment.

Real-world evidence has provided important complementary insights beyond clinical trials. The TRUCKEE study demonstrated that eyes switched from brolucizumab to faricimab experienced anatomical improvement and functional stabilization, suggesting therapeutic flexibility is valuable in cases of suboptimal response or safety concerns [2]. Such data underscore the importance of adaptive treatment strategies in routine practice.

2.2 Imaging Biomarkers and Prognostic Indicators

Advanced retinal imaging has become integral to personalized treatment planning. Structural biomarkers such as ellipsoid zone (EZ) integrity, central retinal thickness, and fluid compartmentalization influence visual prognosis. A Phase III trial analysis demonstrated that preserved EZ continuity correlates with superior visual outcomes, whereas persistent fluid patterns may predict poorer functional recovery [3]. These findings emphasize the need for detailed OCT-based assessment when determining treatment intervals or switching strategies.

2.3 Therapies Beyond Conventional Anti-VEGF

Despite the success of anti-VEGF therapy, limitations include frequent injection schedules and incomplete response in certain patients. Emerging therapies aim to enhance durability and broaden mechanistic targets. Novel pharmacologic strategies include dual-pathway inhibitors and extended-release systems designed to maintain therapeutic drug levels over longer intervals [4]. Future innovations may combine anti-angiogenic and anti-inflammatory mechanisms. As disease chronicity increases, reducing cumulative injection burden while maintaining efficacy is critical for long-term visual preservation and patient adherence.

3. Geographic Atrophy

3.1 Pathophysiology and Therapeutic Rationale

Geographic atrophy represents advanced non-neovascular AMD and is characterized by progressive loss of photoreceptors, retinal pigment epithelium (RPE), and choriocapillaris. Complement system dysregulation plays a pivotal role in GA progression, supported by genetic and molecular evidence implicating alternative pathway activation. Complement inhibition has therefore emerged as a rational disease-modifying strategy.

3.2 Avacincaptad Pegol and the GATHER Trials

Avacincaptad pegol is a complement C5 inhibitor designed to prevent downstream membrane attack complex formation and inflammatory tissue injury. The GATHER1 trial demonstrated a statistically significant reduction in GA lesion growth over 18 months compared with sham treatment [5]. Importantly, lesion growth rate reduction suggests modification of the underlying degenerative process rather than purely symptomatic benefit. Subsequent summaries of GATHER1 and GATHER2 confirmed reproducibility of lesion growth attenuation and supported regulatory approval considerations [6]. The therapeutic effect appears consistent across lesion subtypes, although long-term visual function preservation requires continued study. A comprehensive review further elaborated on pharmacodynamics, dosing strategies, and clinical outcomes of avacincaptad pegol, reinforcing its role as a milestone in GA management [7]. Broader reviews of emerging GA therapies position complement inhibition at the forefront of current disease-modifying interventions [8].

3.3 Safety Profile of Complement Inhibition

While complement inhibition represents a major therapeutic advance, safety monitoring remains essential. Reviews of newly approved AMD therapeutics note a modest increase in conversion to neovascular AMD among treated GA patients, necessitating vigilant OCT surveillance [9]. Nevertheless, intraocular inflammation rates appear manageable, and the overall benefit–risk profile supports clinical use in appropriately selected patients. Long-term post-marketing data will be critical to refine patient selection criteria and optimize follow-up intervals.

4. Gene Therapy in AMD

Gene therapy seeks to provide sustained intraocular production of anti-angiogenic proteins via viral vector delivery. By enabling endogenous expression of therapeutic agents, gene-based strategies aim to reduce or eliminate the need for repeated intravitreal injections. A comprehensive review of gene therapies for AMD described adeno-associated viral vectors, subretinal versus intravitreal delivery routes, and early-phase clinical findings [10]. Although challenges remain—including immunogenicity, vector durability, and surgical considerations—gene therapy represents a potentially transformative modality. In the future, gene-based complement modulation strategies may also be explored for GA, extending the concept of durable disease modification beyond neovascular AMD.

5. Lifestyle Modification and Systemic Interventions

5.1 Modifiable Risk Factors

AMD progression is influenced by both genetic and environmental factors. Smoking remains the strongest modifiable risk factor. Dietary habits, obesity, and cardiovascular health also contribute to disease trajectory. Recent evidence emphasizes structured counseling on smoking cessation, dietary quality, and systemic risk control as part of comprehensive AMD management [11]. Integrating preventive strategies into ophthalmic practice may slow progression, particularly in intermediate stages.

5.2 Nutritional Supplementation and Microbiome Interactions

AREDS-based supplementation remains recommended for patients with intermediate AMD or advanced disease in one eye. Updated analyses reaffirm the benefit of antioxidant and zinc-based formulations in delaying progression [12]. Emerging research suggests that supplementation effects

may interact with genetic risk profiles and influence intestinal microbiome composition in advanced AMD [13]. These findings highlight the complex systemic interplay underlying retinal degeneration and suggest future personalized nutrition strategies may become feasible.

6. Inflammation and Cytokine Pathways

Inflammatory signaling contributes significantly to AMD pathogenesis. Cytokines such as interleukins, TNF-α, and complement components participate in retinal tissue damage and neovascularization. A recent pharmacologic review detailed cytokine-mediated mechanisms and proposed potential therapeutic targets beyond VEGF inhibition [14]. Modulating inflammatory cascades may complement existing therapies, particularly in cases demonstrating incomplete response to monotherapy. Combination strategies targeting angiogenesis, complement activation, and cytokine signaling could represent the next generation of multimodal AMD treatment.

7. Integrated Treatment Algorithm

Modern AMD management requires an individualized, stage-specific approach:

- **Neovascular AMD**: Initiate anti-VEGF therapy with biomarker-guided interval adjustment [1–3]. Consider switching strategies for suboptimal responders [2].
- **Geographic Atrophy**: Evaluate eligibility for complement inhibition and monitor for neovascular conversion [5–9].
- **All Stages**: Implement lifestyle counseling and nutritional supplementation when indicated [11–13].

Gene therapy may eventually reduce injection burden and redefine long-term management paradigms [10].

8. Conclusion

The therapeutic landscape of age-related macular degeneration has undergone substantial transformation. Anti-VEGF therapy remains foundational for neovascular AMD, while complement C5 inhibition with avacincaptad pegol represents a landmark advancement for geographic atrophy. Gene therapy, biomarker-guided treatment optimization, inflammatory pathway targeting, and systemic risk modification collectively define a new era of personalized AMD care. Future research should focus on long-term safety, functional outcome preservation, combination strategies,

and integration of genetic and systemic biomarkers into therapeutic algorithms.

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Alzheimer's Disease: A Systemic Review of Recent Substantial Amyloid Beta Disaggregation Therapeutics

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disease associated with impaired cortical as well as cognitive functions. The aggregation of β-amyloid (Aβ) plaques outside and around the nerve cell is one of the reasons which causes AD. The accumulation of Aβ peptides inside the neurons involves in cognitive impairment, synaptic dysfunction and constitution of amyloid plaques. Still no effective treatment has been made which can slow down the progression of these Aβ plaques. Based on various researches, different anti-Aβ drugs have been made to decrease the production of Aβ plaques by inhibiting the β and γ secretase enzymes and also to stimulate the breakdown of preexisting Aβ plaques in the brain. Globally, many drug companies have performed clinical trials on AD patients, based upon amyloid hypothesis, to synthesize anti-amyloid drugs. Many researchers have dismissed the Aβ hypothesis of AD due to the failure of several amyloid-targeted therapies in various clinical trials performed on mild to moderate AD patients, as discussed in this article. Researchers are still developing and testing various therapies for an ideal drug of AD, in ongoing clinical trials. In this article, such anti-amyloid drugs are discussed which can prevent the formation of Aβ oligomer and suppress their toxicity.

Keywords: Alzheimer's disease; β-amyloid; Amyloid cascade hypothesis; APOE4 genotype; Anti-amyloid antibodies; β-secretase inhibitors

1. Introduction

Alzheimer's disease (AD) is the most prevalent type of dementia, a terminology refers to memory loss and other cognitive dysfunction that are severe enough to cause difficulty in daily activities, causing patients and their families enormous suffering. About 50 million people are currently suffering from AD worldwide [1]. After cardiovascular and cerebrovascular disorders and malignant tumors, AD has risen to become the third most frequent cause of disability and mortality among older adults [1]. AD is a neurodegenerative illness results in memory loss and cognitive impairment [2]. Dementia is a disorder marked by progressive decrease in more than two cognitive domains, like memory, language, visuospatial function, behavior, and personality as a result of which the ability to execute essential everyday activities are lost [3]. AD is designated globally as a public health concern by the World Health Organization (WHO). As of yet, no specific treatment has been discovered for this sickness [4].

About a century ago, Alois Alzheimer hospitalized a 51-year-old patient named Auguste D., for growing cognitive impairment. After some years of her death, Alzheimer's histological tests of her brain lead him to the conclusion that he was witnessing a distinct clinical-chronic process, which

was later termed AD [5]. AD is a multifaceted illness that is influenced by a number of factors. The exact pathophysiology of AD is still unknown because of the human brain complexity. Many hypotheses have been proposed for AD, such as the Aβ cascade hypothesis, cholinergic, tau, presenilin, oxidative stress, calcium inflammation hypothesis, and so on. As a result, a lot of work has gone into developing anti-AD medications depending on these hypotheses [6].

At microscopic level, it is studied that the characteristic nodules in AD were also extracellular senile plaques, formed by Aβ peptide reserves, and intraneuronal fibrillary clumps formed by abnormally hyperphosphorylated tau protein in the medial temporal lobe structures as well as cortex brain regions. The mechanism responsible for causing AD is still not entirely known, but hereditary, pathological, and metabolic evidence suggests that gradual manufacturing and subsequent growth of Aβ, a proteolytic subunit of the membrane-associated amyloid precursor protein (APP), plays a very important role. Aβ peptide, the principal factor of neuritic plaque, is generated in excessive proportions by an abnormal breakdown of APP, a membrane-bound glycoprotein whose principal objective is unknown till now, however, it has been linked to neuronal plasticity and neuron synapse formation [7]. The primary physiological pathway of APP digestion in neurons is followed out by α-secretase and γ-secretase to form P3 protein. However, in AD, an amyloid precursor protein pathway is promoted by the breakdown of APP by β-secretase, accompanied by γ-secretase, to form Aβ peptides comprising 39 to 42 amino acid residues. According to biochemical investigations on cell lysates as well as brains, the most prevalent are Aβ 1-40 (90%) and Aβ 1- 42 (10%). Cells in the brain secrete this protein in a dissolved form, which is normally eliminated. However, when aberrant conditions make more APP susceptible to β-secretase cleavage, the Aβ elimination mechanism in the process becomes overwhelmed. This ultimately results in the accumulation of Aβ, which forms oligomeric, multimeric, and fibrillar clumps, causing neurodegeneration. The aggregation as well as deposition of Aβ results in the production of extracellular plaques, which are one of the physiological markers of the illness [8].

2. Amyloid Precursor Protein (APP) and β-amyloid Production

Initially, amyloid protein was isolated and distinguished from the AD patient's cerebral vasculature of brain and thereafter the cloning of APP was done [9]. The cleavage of APP is done with the help of secretases enzyme, as it is a type-I transmembrane protein. The APP gene can be found on chromosome 21 and three major isoforms (or homologs) can be prepared from its mRNA by the process of alternative splicing. Amino acid

homologue i.e. 695 mainly found in neurons, preferentially 751 and 770 homologue amino acids systematically expressed. The Aβ region in the APP is found partly inside the ectodomain and transmembrane region of APP as the peptides of 40-43 amino acid residues. Aβ42 is the disease-associated isoform of Aβ, accounting for 5-10% total amount of Aβ generated [10]. The amyloid plaques which are deposited initially seen in the brains, suffering from AD, are generated due to Aβ42. APP is processed by proteolysis by enzymes α, β, and γ-secretases. At amino acid 17, α-secretase enzyme cleaves the majority of APP in the Aβ domain, which results in the non-generation of Aβ, and it generates soluble αAPP which subsequently released in non-amyloidogenic pathway. APP, on the other hand, is also cleaved by β-secretases and γ-secretases in the Aβ domain at the N-terminus and C-terminus respectively, which results in the generation of Aβ in amyloidogenic mechanism [11]. A larger amount of APP is present in patients suffering with Down syndrome due to the trisomy of chromosome 21. The AD is generally known to grow too soon in people suffering with Down syndrome [12]. In addition, automatic transmission dominant mutations of APP and presenilin associated with contagious forms of premature AD have been demonstrated to increase Aβ output and to generate more Aβ aggregation. So Aβ peptide has become central focus to AD research on the basis of these genetic markers. Aβ was originally created at the plasma membrane and supposed to be released in the extracellular form of Aβ. It is postulated that in AD patients, released Aβ progressively accumulates in extracellular environment till it begins the formation of clumps of β-pleated amyloid fibrils which are insoluble in nature and are made up of 7-9 nm broad antiparallel-pleated sheets of fibres. Several theories suggests that these extracellular Aβ fibrils have a detrimental impact on the neighboring nerve cells and their functions [13]. Recently, it has been proposed that intermediary products of Aβ fibril synthesis, such as Aβ oligomers having lower molecular weight and protofibrils, are extremely harmful to neurons and alter neuroplasticity than Aβ fibrils [14]. Furthermore, soluble Aβ interacts more with brain functions in the AD brain than insoluble Aβ . Protofibrils is a soluble form of Aβ and these soluble Aβ groups are generally assumed to have significant pathogenic impacts [15].

3. Aβ Cascade Hypothesis

The transmembrane protein APP, which is a component of many cell types, including neurons and glial cells, is broken down into peptide Aβ during the proteolysis process. There are several versions of the molecule produced by substitution concatenation in humans, with APP695 being the most prevalent [16][17][18]. The enzyme protein complex -α, -β, and -γ secretase, which is made up of presenilin and nicastrin molecules, cleaves APP into smaller

peptide pieces, one of which is Aβ. When APP is catabolized by α-secretase, two fragments are produced: a soluble sAPPα fragment that stays in the extracellular space and an 83 carboxyterminal amino acid fragment (C83) that is attached to the plasma membrane [19][20][21]. In addition to improving synaptic plasticity, learning, and memory, sAPPα also helps to reduce metabolic stress and regulate neural excitability. APP is selectively cleaved by β-secretase 1 (BACE) in neuropathic conditions, resulting in the fragmentation of APP into sAPPβ and a fraction of 99 amino acids (C99). The peptides Aβ(1-40) and Aβ(1-42), which are produced after further processing of C99 cleavage by γ-secretase, are believed to be responsible for the development of neuritic plaques [22]. According to the amyloid cascade hypothesis, the pathophysiology of AD, which results in neurotoxicity and neuronal degeneration, is heavily influenced by the production, aggregation, and deposition of Aβ peptides, particularly Aβ(1-42) [23]. The development of fibrillar plexuses and enhanced Tau phosphorylation are two additional effects of excessive extracellular Aβ. The amyloid cascade hypothesis, however, falls short in describing the fundamental reasons of rare AD, where formation of Aβ is unlikely to have a clear hereditary basis [24][25].

3.1. Antiamyloidogenic Pathways as Potential Therapeutic Targets for Modifying the Progression of AD

The pharmaceutical industry has mostly concentrated on amyloid-centric approaches during the past 20 years, investing significant resources to create effective AD medicines. Experts have questioned the strategy's practicality, nevertheless, in light of the numerous clinical trial failures of drug candidates [21][22][25]. Lack of biomarkers that can reliably identify AD in its early stages is one likely cause of failure. The people who are currently being enrolled in phase III studies appear to be suffering from advanced stages of AD, rendering any suggested treatment ineffective. New diagnostic tools which are able to do early detection are therefore critically needed. Meanwhile, numerous new medications that target the mechanism of amyloid production are continually being developed. The inhibition of γ- and β-secretase can lower the Aβ production from APP, and increasing α-secretase activity must be taken into account [26].

3.1.1. β-Secretase Inhibitors and its Modulators

The amyloidogenic APP-processing pathway's early stages involve the β-secretase enzyme complex. Because this complex also includes multiple additional substrates in addition to the APP, it is challenging to make β-secretase inhibitors. For instance, β-secretase's ultimate target is neuregulin-1, which is crucial for CNS axon myelination and synaptic plasticity [18]. Even if the enzyme is specifically inhibited, a wide range of substrates might

cause considerable adverse effects. Nonetheless, MK8931 (clinical trial ID# NCT01739348), E2609 (NCT01600859), and LY2886721 (NCT01807026 and NCT01561430) have all showed effectiveness in reducing Aβ production in the cerebrospinal fluid (CSF) by up to 80–90% in people. So yet, no β-secretase inhibitors have reached the market [27][28][29][30].

3.1.2. Inhibitors of γ-Secretase and its Modulator

The production of Aβ(1-40) and Aβ(1-42) is the result of the final step of amyloidogenesis, which is carried out by the γ-secretase complex. Inhibiting -secretase was once thought to be a potential method for treating diseases. On the other hand, β-secretase inhibitors have similar problems with substrate promiscuity. Notch protein controls cell multiplication, differentiation, growth and cell transmission, is primary targets of γ-secretase [31]. Similar to β-secretase inhibitors, side effects like off-target are a big worry [32].

γ-secretase inhibitor like semagacestat (LY450139) decreases the levels of Aβ in human blood and CSF [33]. Results of clinical trials (NCT00762411, NCT01035138 and NCT00762411) recruited more than 3000 patients an example of the worst possible consequences. It has been reported that treatment with semagacestat was associated with exacerbations of the ability to carry out daily life activities of AD patients (ADAS-cog scale). Moreover, side effects include weight loss, more risk of skin cancer and infection. Avagacestat is one more example of γ-secretase inhibitor discontinued due to lack of efficacy [34][35][36].

The selective γ-secretase modulator (SGSM), which is linked to significant enzyme inhibition, is theoretically created to prevent undesirable outcomes. Therefore, the goal of these treatments is to prevent the processing of the APP from occurring without the use of other signalling pathways, including Notch [37]. The discovery that several non-steroidal anti-inflammatory medicines (NSAIDs) reduced Aβ peptide (1-42) levels in vitro and in vivo was the catalyst for the development of SGSM. Ibuprofen, sulindac and flurbiprofen are a few examples of this category of medication [38]. The commonly accepted mechanism of action (MOA) for NSAIDs is the suppression of the cyclooxygenase (COX) enzyme. R-flurbiprofen (Tarenflurbil), unlike ibuprofen, does not block the COX enzyme, hence its ability to lower levels of Aβ cannot be attributed to this. Sadly, in their respective clinical studies, tarenflurbil and ibuprofen failed to demonstrate efficacy for the treatment of AD [38][39]. CHF5074 is an NSAID without COX inhibitory action, just like R-flurbiprofen. By impeding the γ-secretase complex, CHF5074 prevented the formation of Aβ(1- 42) in vitro [40][41][42]. This substance has been labelled as a microglial modulator in recent studies due to its ability to decrease amyloid burden and microglial

activation. The results of a phase II trial in people with moderate cognitive impairment (MCI) show that CHF5074 therapy enhances a number of cognitive tests and lowers inflammatory marker levels in the CSF. Interest in NSAIDs as a medicine relatively effective for decreasing Aβ(1- 42) levels was inspired by the prospect that long-term NSAID usage would provide some protection against AD. However, negative outcomes from NSAID-related clinical research demonstrate that this idea has to be improved further [43].

NIC5-15 is another probable instance of SGSM. It is a chemical that is found in nature and is also referred to as pinitol. NIC5-15 is a natural cyclic sugar alcohol [40]. It is reported that this compound can probably regulate γ-secretase and also reduce the production of Aβ without affecting the Notch in substrate cutting. Because there is no any reviewed data, therefore, for this chemical, any stated outcomes should be regarded as a statement of future prospects that need hard scientific evidences. However, the compound is asserted to have enhanced memory and cognitive performance in a model of preclinical AD neurological pathology. These results suggest that NIC5-15 might serve as a promising therapeutic agent to use in the treatment of AD for two distinct reasons: (a) helps to retain Notch activation, and (b) it has the potential to be an insulin sensitizer, assuming the data are accurate. It should also be the subject of research to serve as an anti-inflammatory inhibitor because it can stop microglia from activating. Yet additional researchers have not yet confirmed these results [26].

3.2. Inhibition of β-Amyloid Peptides Aggregation

Aggregation of Aβ peptides results in the formation of amyloid plaques. To inhibit the production of senile plaques, the following chemicals were produced.

3-amino-1-propaneosulfonic acid (3-APS, Alzhemed, tramiprosate) is the only Aβ aggregation inhibitor that has progressed to phase III studies. This medication was developed to interfere with or inhibit the interaction of soluble Aβ with endogenous glycosaminoglycans. It has been discovered that glycosaminoglycans promote the synthesis and deposition of Aβ amyloid fibrils. However, this chemical has been banned in Europe as a result of the phase III clinical trial unsatisfactory results in 2007 [44].

A proline-rich polypeptide complex i.e. colostrinin present in human colostrum, ovine, and bovine, lowers Aβ-aggregation and neurotoxicity in cell experiments and improves memory retention in numerous mice models. After 15 months of therapy, participants in a phase II trial who had moderate AD showed modest improvements on the mini mental state examination, but these positive effects were not maintained after another 15 months of ongoing therapy [45].

Scyllo-inositol (ELND005) is another example of amyloid anti-aggregation drug that may be taken orally that reduces Aβ toxicity in the hippocampus of mouse. Patients with mild to moderate stages of AD participated in a phase II clinical trial which lasted for 18-months using ELND005. The major clinical efficiency objectives of this trial weren't accomplished [46].

The metal-chelating 8-hydroxiquinolines (8-HQ) molecules clioquinol and PBT2 were also used in clinical trials for the treatment of AD [47]. Although the precise mode of action is uncertain, it is thought that these kinds of molecules prevent base metals from attaching to the A peptides of brain. It has been suggested that copper ions sticking to A, which results in the metal-mediated production of reactive oxygen species (ROS), may contribute to the higher levels of oxidative stress found in the brains of AD patients [48]. Additionally, it was suggested that 8-HQs could balance out the amounts of copper and zinc in cells while preventing Aβ aggregation. Unfortunately, due to lack of efficiency, these compounds failed in clinical development phase II and III [26].

3.3. Aβ Aggregates Removal Compounds

Another possible amyloidogenic pathway-based therapy strategy is promoting the eradication of already existing aggregates as well as plaques. Three distinct tactics have been tested in order to accomplish this.

3.3.1. Transmission Modulation of Aβ Between the Peripheral Circulation and the Brain

The transmission of Aβ between the peripheral circulation and the CNS is done by low density lipoprotein receptor-related protein (LRP-1), apolipoproteins (APOE) and receptor for advanced glycation end products (RAGE). The main function of LRP-1 is to increase the Aβ discharge from the brain to blood whereas RAGE enhances Aβ transmission throughout the blood-brain barrier [49][50][51]. Any therapy based on this strategy aims to minimize cerebral amyloid burden by aiming to limit Aβ to the peripheral circulation. A variety of techniques, including the peripheral delivery of LRP-1, have been proposed to achieve this goal. Yet, RAGE inhibitors and modulators are the sole therapeutic candidates which have progressed to the clinical stage. They consist of TTP4000, which successfully completed its phase I trial, and PF-0449470052, which did not succeed in phase II trials. The findings of this experiment have not been made public [51].

3.3.2. Degradation of Amyloid Plaques by Enzyme Activation

The degradation of aggregates and amyloid plaques is aided by a number of proteases, particularly plasmin, IDE, neprilysin, angiotensin converting enzyme, endothelin transforming enzyme and metalloproteinases. In AD,

there is a reduction in the protein levels associated with these enzymes, which causes Aβ to be produced and deposited. Absolutely no drugs with this MOA progressed to advanced therapeutic uses, despite being a convincing method for creating disease-modifying medications due to a lack of specificity [24][52].

3.3.3. Anti-Amyloid Immunotherapy

Active immunotherapy is the immunotherapy technique intended to improve Aβ elimination in AD in order to lessen the amyloid burden. In transgenic AD mouse models, the active immunization is effectively tested with Aβ(1-42) or alternative synthetic components. Assays are often predicated on activating microglia's phagocytic capability in order to stimulate immunological responses, T cells and B cells. Human testing was originally encouraging, however, treatment with the very first vaccination (AN1792) resulted in substantial side effects, forcing the phase II study to be halted. AN1792 was made up of a synthetic Aβ(1-42) peptide and an adjuvant known as QS-21. 6% of patients experienced cerebral inflammation brought on by a T cell-mediated autoimmune reaction, which result in aseptic meningoencephalitis [26].
Second-generation vaccinations were developed employing a condensed Aβ(1-6) peptide sequence to prevent the nonspecific immune response observed with the full-length immunization. The second-generation vaccine to enter the clinical stages of development was CAD 106, created by Novartis, for the first time [53]. A phase II clinical trial found that 75% of the participants treated had an Aβ-specific antibody response without triggering any unfavorable inflammatory reactions. Janssen developed a drug ACC-001 which have finished two phase II studies (NCT00479557 and NCT01284387), while a third phase II trial (NCT01227564) remain in progress. The pharmaceutical industry nevertheless has given up on further developing this vaccine. There are several other vaccines in various phases of preclinical advancement, particularly tetra-palmitoylated Aβ(1-15) which is remanufactured in a liposome (ACI-24), AF205 and MER5101 [54][55][56][57].

Passive immunotherapy constitutes the delivery of monoclonal or polyclonal antibodies targeting Aβ. Anti-Aβ antibodies are administered intravenously to the patient as part of this treatment. The absence of proinflammatory T cell-mediated immune responses is a benefit of this method over active vaccination. Passive vaccination decreases cerebral amyloid burden and enhances cognition in transgenic rats, despite the fact that the number of amyloid plaques is not greatly decreased. The reason for this might be explained by soluble amyloid oligomers elimination, that are now known to be vital components along the pathophysiologic chain of AD [26].

Monoclonal antibodies which have successfully reached relatively advanced phases in their clinical trials are bapineuzumab and solanezumab [58]. Two phase III clinical investigations, though, were unsuccessful in 2012 because they were ineffective for AD patients. Targeting Aβ(1-6) and Aβ(12-28), bapineuzumab and solanezumab are humanizing monoclonal antibodies respectively. Bapineuzumab did not significantly improve cognitive function throughout clinical trials, despite being reported to significantly reduce brain amyloid deposits and phosphorylated Tau in CSF fluid. AD patients who participated in a research trial were given 400 mg of solanezumab or a placebo once each month for 80 weeks. Statistical significance was not achieved in this study, despite the fact that the results revealed that solanezumab may enhance cognitive function in those with moderate Alzheimer's disease. The effectiveness of solanezumab in treating AD in patients as well as older people with normal cognitive function who may be at risk of getting AD as they age is now being investigated in phase III clinical trials [59][60].

Researchers are currently studying gantenerumab, another monoclonal antibody, in individuals who have genetic abnormalities that put them at risk of developing presenile AD. In one clinical trial (NCT01760005), the gantenerumab and solanezumab efficacy was assessed in the early stages of the illness. Additionally, two further phase III trials are being conducted to evaluate the effectiveness of gantenerumab in people with mild to moderate AD. A completely human-derived IgG1 antibody called gantenerumab has been created to attach to Aβ fibres' structural epitope with great affinity. The recruitment of microglia and subsequent phagocytosis will probably result in amyloid plaque destruction. This idea is supported by experimental research in transgenic mice [26][61].

There have been several monoclonal antibodies created to target Aβ, such as PF-04360365 (ponezumab), MABT5102A and GSK933776A. PF-04360365 is a drug that targets amino acids 33–40 at the free carboxy terminal of the Aβ peptide. Meanwhile, MABT5102A has a strong binding affinity for Aβ monomers, fibrils and oligomers. On the other hand, GSK933776A attaches to the N-terminal Aβ(1–5) and shares similarities with bapineuzumab. These medications have diverse methods of action and target various parts of the Aβ peptide, which may have an impact on their efficacy and safety profiles. Additionally, numerous additional passive immunotherapies, including NI-101, SAR-228810, and BAN-2401, are in phase I clinical studies [56][57][61]. Monoclonal antibody crenezumab, often referred to as MABT5102A, has an IgG4 backbone. In April 2014, a phase II clinical trial examining the security and effectiveness of crenezumab in people with AD was finished. A recently completed phase II trial, which started in November 2013, is testing crenezumab's effectiveness as well as safety in E280A

carriers having no symptoms of the autosomal-dominant PSEN1 mutation [62].

The large-scale clinical research with aducanumab, known as the EMERGE study, demonstrated the efficacy of amyloid targeting in individuals with AD. In the treatment of early-stage AD, the anti-amyloid antibody BAN2401, which targets soluble Aβ protofibrils, has also produced encouraging results. Phase 2 of BAN2401's trial is complete, and phase 3 testing is already taking place. ALZ-801, an oral medication, has also been demonstrated to entirely halt the onset of AD. The effectiveness of ALZ-801 has shown promising results in subgroup analysis of prespecified groups of patients, specifically those with Aβ oligomers but without plaque binding. Patients with an elevated load of A oligomers who are APOE4 carriers seem to respond better to BAN2401 and ALZ-801 than non-carriers [62].

Table 1: Anti-amyloid drugs undergoing clinical trials [63]:

Drug	Study Phases	Sponsor	Start Date	Expected Completion Date	Status (CT.gov ID)
LY3372993	1	Eli Lilly	Jul 2020	Feb 2022	Recruiting (NCT04451408)
Abvac40	2	Araclon Biotech	Feb 2018	Feb 2022	Recruiting (NCT03461276)
ALZ-801	2	Alzheon	Sept 2020	May 2023	Recruiting (NCT04693520)
APH-1105	2	Aphios	Jun 2021	Dec 2022	Not yet recruiting (NCT03806478)
Crenezumab	2	Genentech, NIA Banner Alzheimer's Institute	Dec 2013	Feb 2022	Active, not recruiting (NCT01998841)
Donanemab (LY3002813)	2	Eli Lilly	Dec 2017	Nov 2021	Active, not recruiting (NCT03367403)
Gantenerumab	2	Roche	Dec 2020	Feb 2024	Recruiting (NCT04592341)
IVIG (NewGam 10%)	2	Sutter Health	Jan 2011	Dec 2019	Active, not recruiting (NCT01300728)
Lecanemab (BAN2401)	2	Eisai	Dec 2012	Feb 2025	Active, not recruiting (NCT01767311)

PQ912	2	Vivoryon Therapeutics AG, ADCS, NIA	Jun 2021	Jan 2023	Not yet recruiting (NCT03919162)
RO7126209	2	Roche	Mar 2021	Oct 2024	Recruiting (NCT04639050)
Thiethylperazine (TEP)	2	Immungenetics AG	Nov 2017	Jul 2021	Active, not recruiting (NCT03417986)
Aducanumab	3	Biogen	Mar 2020	Oct 2023	Enrolling by invitation (NCT04241068)
Azeliragon	3	vTv Therapeutics	Jun 2019	Jul 2023	Active, not recruiting (NCT03980730)
Gantenerumab	3	Roche	Mar 2014	Apr 2021	Active, not recruiting (NCT02051608)
		Roche	Jun 2018	Nov 2023	Recruiting (NCT03444870)
		Roche	Aug 2018	Nov 2023	Active, not recruiting (NCT03443973)
		Roche	May 2020	Feb 2023	Recruiting, extension study (NCT04339413)
		Roche	Feb 2021	Dec 2024	Not yet recruiting, extension study (NCT04374253)
Gantanerumab & solanezumab	3	Washington University, Eli Lilly, Roche, NIA, Alzheimer's Association	Dec 2012	July 2022	Recruiting (NCT01760005)
Lecanemab (BAN2401)	3	Eisai, Biogen	Mar 2019	Aug 2024	Recruiting (NCT03887455)
		Eisai, Biogen, ACTC, NIA	Jul 2020	Oct 2027	Recruiting (NCT04468659)
Solanezumab	3	Eli Lilly, ATRI	Feb 2014	Jan 2023	Active, not recruiting

					(NCT02008357)

4. Conclusion

In conclusion, data reveals that AD neuropathology is complex and includes several biological processes. Since the amyloid cascade hypothesis has greatly influenced the field for more than two decades, a lot of work has been done on preventing and getting rid of Aβ and senile plaques. Nevertheless, despite this amyloid-centric strategy, AD medicines haven't shown that they significantly improve patients' cognitive function. When creating novel experimental treatments, it is important to keep in mind that dendritic spine defects are a contributing component to the cognitive impairment found in this condition. To better comprehend the aetiology of AD, we need take into account activities occurring at the synapse as well as the amyloid cascade theory. It is critical to investigate alternative therapeutic approaches in light of the amyloid immunotherapies and BACE1 program repeated failures. As Aβ oligomers are frequently cited as major factors in AD pathology, inhibitors of these oligomers may prove to be successful AD treatments. Clinical trials may be more successful if they target APOE4 carriers in the early stages of disease and use drug concentrations that decrease Aβ oligomers in verified CSF testing.

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Novel Inflammatory Pathways and Biomarker-Focused Targets in Atherosclerosis

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1. INTRODUCTION

Atherosclerosis remains the leading cause of mortality and morbidity worldwide, despite decades of intensive research and revolutionary advances in lipid-lowering therapies. Traditionally simplified as a passive lipid-storage disease, atherosclerosis is now redefined as a complex, multi-dimensional immunometabolic phenomenon situated at the intersection of vascular biology, immunology, and systems genetics [1].

Cardiovascular medicine is currently transitioning from an LDL-centric era to an era of Immunocardiology, where inflammation is recognized as the definitive driver at every stage of the disease. This chapter argues that the atherosclerotic process is not merely a localized narrowing of the arterial lumen but a systemic ecosystem disorder. Within this context, we present a different perspective that integrates the synergistic mechanisms of endothelial priming, cellular plasticity, and cytokine signaling.

The pathogenesis of coronary atherosclerosis is shaped by a vast spectrum of factors, ranging from the physical stress of blood flow (mechanotransduction) to the metabolic outputs of the gut microbiota (TMAO) and the genetic mutations within the bone marrow known as Clonal Hematopoiesis of Indeterminate Potential (CHIP) [2], [3], [4]. These complexities manifest clinically as Residual Inflammatory Risk (RIR), providing the strongest explanation for why patients with LDL-C levels at goal continue to experience major adverse cardiovascular events [5].

The primary objective of this chapter is to consolidate the molecular foundations of atherosclerosis while illuminating the through the lens of recent high-impact literature[6]. By exploring the journey from the initial endothelial priming to the phenotypic identity crisis of vascular smooth muscle cells, this work aims to outline the strategies required to transform atherosclerosis into a preventable condition. Rather than focusing solely on classical statin or ACE inhibitor therapies, this chapter emphasizes precision medicine applications, including RNA-based therapies, gene editing, and targeted inflammation modulation.

2. Molecular Pathways of Atheroinflammation

Atherosclerosis is essentially a chronic, maladaptive inflammatory response of the innate immune system to subendothelial lipoprotein infiltration [2]. While the fundamental role of hypercholesterolemia is well-established, modern pathology highlights that the transition from a fatty streak to a clinically significant lesion involves complex cellular interactions that remain partially "unknown" in human subjects compared to experimental models [6].

The pathogenesis of coronary atherosclerosis is no longer viewed through a reductionist "lipid-only" lens. Instead, it is understood as a three-dimensional regulatory process where mechanical forces, metabolic dysregulation, and synergistic immune signaling converge to drive vascular damage. This section describes the molecular interplay between lipid oxidation, oxidative stress, and the activation of innate immune pathways in atherosclerosis.

2.1. Mechanotransduction and Endothelial Priming

The initiation of atherosclerosis involves a priming phase that often precedes significant lipid deposition.

2.1.1. Mechanical Priming

In human physiology, diffuse intimal thickening (DIT) occurs at arterial branch points due to disturbed blood flow and low shear stress. These mechanical forces are sensed by endothelial mechanoreceptors (e.g., Piezo1), which trigger a pre-lesional inflammatory state by activating the Nuclear Factor-kappa B (NFKB) pathway [6].

2.1.2. Endothelial Activation

This mechanical priming upregulates adhesion molecules such as Vascular Cell Adhesion Molecule-1 (VCAM-1) and Intercellular Adhesion Molecule-1 (ICAM-1) and chemokines (MCP-1), creating a receptive environment for the subsequent retention and oxidation of LDL particles [2].

2.2. Lipid Metabolism and Cellular Plasticity

A pivotal factor recently elucidated in human pathology is that the cellular landscape of the plaque is far more plastic than previously assumed.

2.2.1. The Identity Crisis of VSMCs

While macrophage-derived foam cells are well-known, recent lineage-tracing studies and pathological evidence suggest that a substantial proportion of foam cells in human lesions originate from Vascular Smooth Muscle Cells (VSMCs) [7], [8], [9].

2.2.2. Lipotoxicity and ER Stress

Excessive accumulation of free cholesterol within these cells induces Endoplasmic Reticulum (ER) Stress. This metabolic failure not only drives the transdifferentiation of VSMCs into macrophage-like phenotypes but also serves as a potent endogenous signal for the activation of the Nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3 (NLRP3) inflammasome [10].

2.2.3. NLRP3 Activation

Known as the central protagonist in the inflammatory cascade and its activation within macrophages is a process that ensures an immune response. Systemic triggers such as oxidized LDL (ox-LDL) or tumor necrosis factor-alpha (TNF-alpha) bind to Toll-like receptors (TLRs), activating the Nuclear Factor-kappa B (NF-kappaB) pathway [11]. This stage is essential for the transcriptional upregulation of the NLRP3 scaffold and the precursor pro-interleukin-1beta (pro-IL-1beta) [10].

Internalized cholesterol crystals induce lysosomal rupture, leading to the leakage of Cathepsin B and the efflux of intracellular potassium (K+) [12]. This ionic shift promotes the assembly of the NLRP3-ASC-caspase-1 complex, which proteolytically matures pro-IL-1\beta and pro-IL-18 into their active, highly potent pro-inflammatory forms [13].

2.2.4. Oxidative Stress and the eNOS Uncoupling

The perspectives on vascular damage identifies Nitric Oxide (NO) bioavailability as the primary gatekeeper of endothelial health. In a healthy state, eNOS produces NO, which maintains vasodilation and inhibits platelet aggregation. However, in the atherogenic microenvironment, this protective mechanism is subverted.

2.2.5. The Mechanism of eNOS Uncoupling

Under conditions of high oxidative stress, reactive oxygen species (ROS), primarily superoxide (O_2^-) produced by NADPH Oxidase (NOX), oxidize the essential eNOS cofactor Tetrahydrobiopterin (BH_4) into its inactive form, BH_2. Without BH_4 , the eNOS enzyme undergoes a structural uncoupling. Instead of transferring electrons to L-arginine to produce NO, the uncoupled eNOS transfers them directly to oxygen, thereby becoming a major source of O_2^- itself [14]. The remaining NO rapidly reacts with the newly formed superoxide to create Peroxynitrite ($ONOO^-$), an extremely potent oxidant that induces protein nitration and DNA damage within the vessel wall [15].

While the biochemistry of eNOS is well-understood, its regulation by mechanical forces a critical focus. Shear stress and NO; laminar flow induces eNOS phosphorylation and NO production. In contrast, the disturbed flow leads to chronic eNOS uncoupling, which serves as the fundamental molecular event of endothelial priming [6]. This local failure of the NO system allows for the upregulation of NF-kappaB and the subsequent recruitment of inflammatory cells.

Briefly, under conditions of persistent oxidative stress, the oxidative depletion of BH_4 results in the structural uncoupling of eNOS, transforming the enzyme from a vasoprotective NO producer into a superoxide-generating source, a phenomenon that significantly exacerbates intimal damage.

2.3. Cytokine Synergy and Systemic Integration

The final dimension of inflammatory regulation is the cytokine synergy, where local vascular signals are amplified into a self-sustaining systemic response.

2.3.1. Positive Feedback Loops

IL-1 beta and TNF-alpha act synergistically; TNF-alpha enhances the expression of IL-1 receptors, thereby sensitizing the vascular wall to even low levels of IL-1beta [13].

The IL-6/CRP Axis: This cytokine synergy drives the production of Interleukin-6 (IL-6), which stimulates hepatic synthesis of high-sensitivity C-reactive protein (hs-CRP). This axis represents the "Residual Inflammatory Risk" (RIR), which persists as a driver of cardiovascular events even when LDL levels are pharmacologically controlled [16], [17], [18].

2.3.2. Macrophage Heterogeneity and the M1/M2

While traditional models categorized macrophages as either pro-inflammatory (M1) or anti-inflammatory (M2), recent single-cell RNA sequencing has revealed a much more complex spectrum of phenotypes within the human plaque.

M1-like Macrophages (Pro-inflammatory): These cells are predominantly found in unstable regions of the plaque. They express high levels of pro-inflammatory cytokines such as IL-1beta, IL-6, and TNF, and are characterized by the activation of the NLRP3 inflammasome.

M2-like Macrophages (Resolution-oriented): These cells promote tissue repair and lesion stability. They are involved in the efficient clearance of apoptotic cells, which prevents secondary necrosis and the expansion of the necrotic core.

CD38 and Immunoaging: In aging plaques, M1-like macrophages exhibit increased expression of CD38, an enzyme that degrades NAD^+, leading to metabolic dysfunction and further accelerating vascular aging.

2.3.3. T-Cell Subsets

T-lymphocytes are present at all stages of atherosclerosis as orchestrating the immune response and interact directly with macrophages to modulate the inflammatory environment.

Pro-atherogenic T-cells (Th1): The most abundant subset in lesions, Th1 cells secrete Interferon-gamma (IFN-gamma), which promotes plaque growth and destabilizes the fibrous cap by inhibiting collagen production.

Athero-protective T-cells (Treg): Regulatory T-cells (Tregs) secrete anti-inflammatory cytokines (IL-10, TGF-beta) and promote macrophage efferocytosis, showing a negative correlation with disease progression.

The Th2 Balance: Th2 cells secrete IL-5 and IL-13, which are generally considered protective against atherosclerosis.

2.4. Pyroptosis and Plaque Rupture

The culmination of these integrated mechanisms is Pyroptosis a Caspase-1-mediated inflammatory cell death.

2.4.1. Pathological Discrepancy

While experimental mouse models seldom exhibit spontaneous plaque rupture, human advanced lesions are characterized by extensive pyroptosis and "Vasa Vasorum" neovascularization, leading to intraplaque hemorrhage and sudden fibrous cap destabilization (Fan & Watanabe, 2022). This explosive release of intracellular contents further amplifies the cytokine synergy, creating a pro-thrombotic environment [11].

2.5. Systemic Amplification and the Gut-Vessel Axis

Modern research emphasizes that the integrated mechanisms of atherosclerosis extend beyond the vasculature.

2.5.1. Clonal Hematopoiesis (CHIP)

Somatic mutations in hematopoietic stem cells lead to the emergence of hyper-inflammatory leukocyte clones. These cells exhibit an exaggerated NLRP3 response, providing a molecular explanation for high Residual Inflammatory Risk in elderly patients despite optimal lipid control[3].

2.5.2. Metabolic Priming via the Gut Axis

The gut microbiota-derived metabolite TMAO (Trimethylamine N-oxide) acts as a systemic primer of vascular inflammation. TMAO directly promotes eNOS uncoupling and amplifies NLRP3 assembly in endothelial cells, bridging the gap between diet and the regulation of atherosclerosis [4], [6].

Under disturbed flow conditions, YAP/TAZ translocation to the nucleus promotes the expression of JNK and NF-kappaB, effectively priming the endothelial layer for lipid-induced damage [6].

3. Clinical Biomarkers of Inflammatory Regulation

The clinical translation of integrated mechanisms, endothelial priming, lipid metabolism, and cytokine synergy, requires a multi-modal biomarker approach. Modern biomarkers serve as precision medicine instruments to identify RIR and guide targeted pharmacological interventions.

3.1. Markers of Systemic Cytokine Synergy

As established above the IL-1beta/IL-6/CRP axis is the primary driver of systemic inflammation and the most validated target for clinical risk assessment.

3.1.1. High-Sensitivity C-Reactive Protein (hs-CRP)

While a "known" biomarker, its role has evolved from a general marker of inflammation to a specific tool for identifying candidates for anti-inflammatory therapy. Patients with low LDL-C but hs-CRP > 2 mg/L represent the core RIR population [5].

3.1.2. Interleukin-6 (IL-6)

Moving "upstream" in the cascade, IL-6 has emerged as a superior predictor of long-term mortality and cardiovascular events compared to hs-CRP. It directly reflects the systemic burden of the NLRP3 activation as discussed before [13].

3.2. Indicators of Cellular Plasticity and Plaque Vulnerability

To address the VSMCs and the metabolic stress within the plaque, biomarkers reflecting enzymatic activity and oxidative damage are essential.

3.2.1. Lipoprotein-Associated Phospholipase A2 (Lp-PLA2)

Primarily secreted by macrophages and VSMC-derived foam cells, Lp-PLA2 reflects the degree of lipotoxicity within the plaque. High levels are strongly associated with the expansion of the necrotic core and subsequent fibrous cap thinning [6], [19].

3.2.2. Myeloperoxidase (MPO)

As a marker of neutrophil activation and oxidative stress, MPO levels indicate the intensity of eNOS uncoupling and endothelial damage [15].

3.2.3. Next-Generation Biomarkers

Alongside classic inflammation markers, new generation biomarkers such as Growth Differentiation Factor 15, a common indicator of myocardial stress and systemic inflammation, has high prognostic value, especially in atherosclerotic patients with concomitant atrial fibrillation and heart failure. And sCD40L, reflecting T-cell/platelet activation, used to predict the risk of atherothrombosis.

3.3. Metabolomic and Epigenetic Biomarkers

New biomarkers are bridging the gap between systemic signals and local vascular events.

3.3.1. Trimethylamine N-oxide (TMAO)

Reflecting the Gut-Vessel Axis, TMAO serves as a metabolic biomarker of systemic priming. Elevated levels identify individuals where diet-microbiota interactions are actively driving NLRP3-mediated vascular inflammation [4].

3.3.2. miRNAs (Circulating microRNAs)

The integration of circulating microRNAs (miRNAs) into the biomarker landscape offers a window into the epigenetic regulation of atherosclerosis that systemic proteins cannot provide. miRNAs are small non-coding RNAs that function as post-transcriptional regulators, and their stability in the circulation often encapsulated in exosomes makes them ideal candidates for liquid biopsies. Consistent with the Identity Crisis of VSMCs, the downregulation of miR-143/145 (markers of VSMC health) clusters reflects the phenotypic switch from a contractile to a pro-inflammatory synthetic state and miR-92a (marker of endothelial priming) provide the artery wall's epigenetic state [20], [21]. Conversely, elevated levels of miR-21 and miR-221 serve as indicators of active vascular remodeling and foam cell formation. These non-coding RNAs offer a window into cellular plasticity long before systemic protein levels rise[22], [23]. miRNAs such as miR-92a and miR-126 are highly sensitive to mechanical shear stress. Their altered expression patterns can identify endothelial priming long before hs-CRP elevations or visible plaque formation on imaging, providing a tool for primordial prevention. By combining microRNA profiles with traditional markers like hs-CRP, clinicians can achieve a risk profile that integrates genetic susceptibility, epigenetic status, and systemic inflammatory burden[10].

3.3.3. CHIP (Clonal Hematopoiesis) Monitoring

The detection of somatic mutations (e.g., *TET2*) in peripheral blood leukocytes identifies a unique high-risk subset of patients whose inflammation is genetically driven and potentially resistant to traditional therapies [3].

3.4. Multi-Omic Integration for Precision Cardiology

The future of cardiovascular risk assessment lies in the integration of these markers. By combining genetic (CHIP), epigenetic (miRNA), and metabolic (TMAO) data with classic inflammatory proteins (IL-6), clinicians can move beyond binary risk models to a truly personalized inflammatory profile. Furthermore, studies highlighting the discrepancy between systemic markers and local plaque events suggest that this gap can be closed with Genetic Risk Scores and Imaging in Clinical Prediction [6].

Polygenic Risk Scores (PRS) integrate thousands of genetic variants to predict an individual's inherent inflammatory set-point. This helps identify patients with high priming susceptibility who may require aggressive early intervention.

Correlating systemic inflammation markers (biomarkers) with metabolic plaque activity detected by PET-CT or fibrous vessel thickness detected by OCT (Optical Coherence Tomography) is important in predicting a patient's risk of rupture. Integration of biomarkers with this imaging data will be the clearest criterion for determining which patient needs aggressive anti-inflammatory treatment (Ziltivekimab, etc.). Utilizing CT-angiography, Perivascular Fat Attenuation Index (FAI) measures the spill-over of inflammatory signals from the coronary artery into the surrounding fat tissue. This provides a three-dimensional view of local vascular inflammation that systemic biomarkers might miss.

4. Targeted Pharmacological Therapies

The therapeutic landscape of atherosclerosis has undergone a paradigm shift, moving beyond lipid-centric approaches toward Immunocardiology. Contemporary approaches now focus on precision medicine applications such as RNA-based therapies, gene editing, and fine-tuned inflammation modulation, rather than relying exclusively on classical statin or ACE inhibitor regimens. This section reviews current and emerging agents designed to target the specific inflammatory nodes identified in the previous chapters also as in Table 1.

Table 1. Key Inflammatory Pathways, Biomarkers, and Targeted Therapies in Atherosclerosis

Pathway / Mechanism	Main Mediators	Representative Biomarkers	Clinical Significance	Candidate Targeted Therapies
Endothelial priming / mechanotransduction	Disturbed flow, Piezo1, NF-κB, VCAM-1, ICAM-1, MCP-1	miR-92a, miR-126	Early endothelial activation and pre-lesional vascular priming	anti-miR-92a, endothelial-stabilizing approaches
Lipid retention and cellular plasticity	ox-LDL, VSMC phenotypic switching, ER stress	Lp-PLA2, decreased miR-143/145	Plaque lipotoxicity, VSMC-to-foam cell transition, and tendency toward necrotic core expansion	Pelacarsen / Olpasiran (Lp(a)-related lipid axis), future VSMC-targeted strategies
NLRP3 inflammasome activation	NLRP3, ASC, caspase-1, IL-1β, IL-18	IL-6, hs-CRP (downstream indicators)	Central mechanism of residual inflammatory risk (RIR)	Colchicine, direct NLRP3 antagonists, canakinumab, ziltivekimab
Oxidative stress / eNOS uncoupling	ROS, NOX, BH4 depletion, peroxynitrite	MPO	Endothelial nitric oxide loss, oxidative injury, and vascular dysfunction	Statins (eNOS recoupling), antioxidant or upstream anti-inflammatory approaches
Cytokine synergy axis	IL-1β, TNF-α, IL-6/CRP axis	hs-CRP, IL-6	Systemic inflammatory burden, cardiovascular event risk, and treatment stratification	Ziltivekimab, canakinumab, colchicine
Plaque vulnerability and thrombo-inflammatory signaling	Neutrophil activation, platelet–T cell signaling	Lp-PLA2, MPO, sCD40L, GDF-15	Thin fibrous cap, elevated atherothrombotic risk, oxidative and immune activation	Anti-inflammatory strategies combined with intensive risk reduction

Pathway / Mechanism	Main Mediators	Representative Biomarkers	Clinical Significance	Candidate Targeted Therapies
Gut–vascular axis	Gut microbiota, CutC/D, TMAO	TMAO	Diet–microbiota-driven systemic priming and amplification of vascular inflammation	TMAO inhibitors
Epigenetic regulation	miR-92a, miR-143/145, miR-21, miR-221	Circulating miRNAs	Endothelial priming, VSMC integrity, and vascular remodeling	anti-miR-92a, miR-145 mimics
Clonal hematopoiesis-driven inflammation	CHIP, TET2-mutant leukocyte clones	CHIP / TET2 status	Genetically driven inflammation that may persist despite conventional therapy	Future gene-editing or CHIP-targeted approaches
Precision lipid / gene-level intervention	LPA mRNA, PCSK9, ANGPTL3	Lp(a), integrated multi-omic risk profile	Genetic lipid burden and long-term cardiovascular risk	ASO/siRNA, CRISPR / base editing

4.1. Colchicine

Once a primitive treatment for gout, low-dose colchicine (0.5 mg/day) has emerged as the first-line anti-inflammatory therapy in cardiovascular medicine. Colchicine non-selectively inhibits tubulin polymerization, but its primary cardiovascular benefit stems from the indirect inhibition of the NLRP3 inflammasome assembly within neutrophils and macrophages [2]. Based on the LoDoCo2 and COLCOT trials, colchicine is recommended in guidelines for patients with persistent RIR despite optimal statin therapy [19], [24], [25].

4.2. Targeted Cytokine Inhibition

While the CANTOS trial with canakinumab (anti-IL-1beta) provided proof-of-concept, the focus has shifted to downstream inhibition of Interleukin-6 for better efficacy and safety[26].

4.2.1.Ziltivekimab

A novel, fully human monoclonal antibody directed against IL-6. Results from the ZEUS trial (2024/2025) have demonstrated that ziltivekimab significantly reduces systemic biomarkers of inflammation (hs-CRP) and major adverse cardiovascular events (MACE) in high-risk patients with chronic kidney disease [3], [5], [27]. Unlike IL-1beta inhibition, targeting IL-6 appears to offer a more robust reduction in the Cytokine Synergy with a potentially manageable infection risk profile.

4.3. Next-Generation Small Molecules

The future of targeted therapy lies in small molecules that can be administered orally, avoiding the cost and administration challenges of monoclonal antibodies.

4.3.1. Direct NLRP3 Antagonists

Several oral inhibitors (e.g., Dapansutrile, MCC950 derivatives) are currently in Phase 2/3 trials. These agents aim to block the inflammasome activation without affecting the priming, theoretically preserving more of the host's innate defense mechanisms [10].

4.4. Precision Lipid Modulation

One of the most significant recent developments is the emergence of therapies targeting Lipoprotein(a), Lp(a), a highly atherogenic particle that is genetically determined and resistant to statins.

Antisense Oligonucleotides (ASOs) and siRNA: Agents such as Pelacarsen (ASO) and Olpasiran (siRNA) target *LPA* mRNA in hepatocytes, reducing plasma Lp(a) levels by up to 80% [1]. These therapies represent a milestone in managing the genetic drivers of atherosclerosis that were previously untreatable.

4.5. CRISPR and Base Editing

Reflecting the Future Frontiers, the application of gene-editing technologies aims to provide permanent solutions for high-risk patients.

4.5.1. One-and-Done Therapy

Experimental CRISPR-Cas9 and base editing techniques are being developed to permanently silence the PCSK9 or ANGPTL3 genes in the liver[28], [29], [30], [31]. This approach could eliminate the need for lifelong adherence to daily medications by achieving a permanent reduction in LDL-C through a single intervention [1]

4.6. Targeting the Gut-Vessel Axis and Epigenetics

4.6.1. TMAO Inhibitors

Small molecules designed to inhibit the gut microbial enzyme (CutC/D) responsible for TMAO production are being investigated to neutralize the Gut-Vessel Axis before vascular priming occurs[4].

4.6.2. MicroRNA Modulators

Synthetic oligonucleotides designed to silence pro-atherogenic miRNAs (e.g., anti-miR-92a) or mimic protective ones (e.g., miR-145 mimics) represent the frontier of Epigenetic Pharmacotherapy, aiming to restore VSMC health and endothelial stability [21].

4.7. Pleiotropic Anti-inflammatory Effects of Standard Therapies

It is crucial to note that modern lipid and glucose lowering agents are, in fact, potent anti-inflammatory modulators:

4.7.1. Statins

Induce recoupling of eNOS and reduce NF-κB activation [15]

4.7.2. SGLT2 Inhibitors:

Reduce NLRP3 activation and oxidative stress, contributing to their remarkable benefits in heart failure and atherosclerosis.

5. Conclusions

The atherosclerosis has evolved from a simple lipid-storage model to a complex system biology framework. As we move toward 2030, the integration of multi-omics, gene editing, and targeted immunomodulation is set to redefine the future frontiers of cardiovascular therapy.

5.1. Systems Genetics and Personalized Risk Prediction

One of the most significant recent development is the shift from single-gene studies to systems genetics.

5.1.1. Gene Regulatory Networks

Future diagnostics will not only look at isolated biomarkers but will map how genetic variations influence entire biological networks across multiple tissues (liver, artery wall, and bone marrow) [1].

5.1.2. Polygenic Risk Scores (PRS)

Combining PRS with systemic inflammatory markers (IL-6, hs-CRP) will allow clinicians to identify high-risk individuals decades before the first plaque appears, enabling primordial prevention.

5.2. Targeting Cellular Heterogeneity and Senescence

Single-cell RNA sequencing (scRNA-seq) has unmasked a previously unknown world of cellular transitions within the plaque.

5.2.1. Senotherapeutics

Targeting senescent cells, those that have stopped dividing but continue to secrete pro-inflammatory cytokines (SASP), represents a novel therapeutic avenue to stabilize advanced plaques and prevent rupture (Björkegren & Lusis, 2022).

5.2.2. Modulating VSMC Plasticity

Since a large portion of foam cells originate from VSMCs, future drugs may focus on re-programming these cells back to their contractile, healthy phenotype rather than just suppressing macrophage activity [1], [6].

5.3. CRISPR and Gene Editing

The most disruptive frontier in cardiovascular pharmacology is the application of CRISPR-Cas9 and base editing.

5.3.1. Permanent Lipid Lowering

Clinical trials targeting the permanent silencing of PCSK9 or ANGPTL3 in the liver offer the promise of a single-injection cure for hypercholesterolemia, effectively eliminating the need for daily statin therapy.

5.3.2. Targeting CHIP

As we better understand Clonal Hematopoiesis (CHIP), gene-editing strategies may eventually be used to neutralize hyper-inflammatory leukocyte clones, addressing a major driver of residual inflammatory risk [2].

5.4. Conclusion

The journey from Endothelial Priming to Cytokine Synergy demonstrates that atherosclerosis is a systemic ecosystem, not just a localized lesion. The successful management of the Residual Inflammatory Risk requires a holistic approach that integrates: Metabolic Control (targeting TMAO and lipids),

Epigenetic Modulation (via microRNAs), Precision Immunotherapy (targeting the NLRP3-IL-6 axis).

In conclusion, the transition from broad-spectrum treatments to precision immunocardiology marks the beginning of an era where atherosclerosis could potentially be managed as a preventable and reversible condition, rather than an inevitable consequence of aging.

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